

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
30 August 2001 (30.08.2001)

PCT

(10) International Publication Number
WO 01/62945 A1

(51) International Patent Classification⁷: **C12N 15/81**,
5/10, 15/01

Baltimore, MD 21208 (US). **KINZLER, Kenneth, W.**
[US/US]; 1403 Halkirk Way, BelAir, MD 21015 (US).

(21) International Application Number: **PCT/US01/05447**

(74) Agents: **KAGAN, Sarah, A. et al.**; Banner & Witcoff,
Ltd., 11th floor, 1001 G Street, N.W., Washington, DC
20001-4597 (US).

(22) International Filing Date: 21 February 2001 (21.02.2001)

(25) Filing Language: English

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(26) Publication Language: English

(30) Priority Data:
60/184,336 23 February 2000 (23.02.2000) US

(63) Related by continuation (CON) or continuation-in-part
(CIP) to earlier application:
US 60/184,336 (CON)
Filed on 23 February 2000 (23.02.2000)

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): **THE
JOHNS HOPKINS UNIVERSITY** [US/US]; Suite 9096,
111 Market Place, Baltimore, MD 21202 (US).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(71) Applicants and

(72) Inventors: **NICOLAIDES, Nicholas, C.** [US/US]; 4
Cider Mill Court, Boothwyn, PA 19061 (US). **SASS,
Philip, M.** [US/US]; 1903 Blackhawk Circle, Audubon,
PA 19403 (US). **GRASSO, Luigi** [US/US]; Apartment
#816, 834 Chestnut Street, Philadelphia, PA 19107
(US). **VOGELSTEIN, Bert** [US/US]; 3700 Breton Way,

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: **METHODS FOR GENERATING HYPERMUTABLE YEAST**

(57) Abstract: Yeast cells are mutagenized to obtain desirable mutants. Mutagenesis is mediated by a defective mismatch repair system which can be enhanced using conventional exogenously applied mutagens. Yeast cells with the defective mismatch repair system are hypermutable, but after selection of desired mutant yeast strains, they can be rendered genetically stable by restoring the mismatch repair system to proper functionality.

WO 01/62945 A1

METHODS FOR GENERATING HYPERMUTABLE YEAST

This application claims the benefit of provisional application serial
5 no. 60/184,336 filed February 23, 2000.

FIELD OF THE INVENTION

The invention is related to the area of mismatch repair genes. In
particular it is related to the field of *in situ* mutagenesis of single celled
10 organisms.

BACKGROUND OF THE INVENTION

Within the past four years, the genetic cause of the Hereditary
Nonpolyposis Colorectal Cancer Syndrome (HNPCC), also known as
15 Lynch syndrome II, has been ascertained for the majority of kindred's
affected with the disease (Liu, B., Parsons, R., Papadopoulos, N.,
Nicolaidis, N.C., Lynch, H.T., Watson, P., Jass, J.R., Dunlop, M., Wyllie,
A., Peltomaki, P., de la Chapelle, A., Hamilton, S.R., Vogelstein, B., and
Kinzler, K.W. 1996. Analysis of mismatch repair genes in hereditary
20 non-polyposis colorectal cancer patients. Nat. Med. 2:169-174). The
molecular basis of HNPCC involves genetic instability resulting from
defective mismatch repair (MMR). To date, six genes have been identified
in humans that encode for proteins and appear to participate in the MMR
process, including the *mutS* homologs *GTBP*, *hMSH2*, and *hMSH3* and the
25 *mutL* homologs *hMLH1*, *hPMS1*, and *hPMS2* (Bronner, C.E., Baker, S.M.,
Morrison, P.T., Warren, G., Smith, L.G., Lescoe, M.K., Kane, M.,
Earabino, C., Lipford, J., Lindblom, A., Tannergard, P., Bollag, R.J.,
Godwin, A., R., Ward, D.C., Nordenskjold, M., Fishel, R., Kolodner, R.,
and Liskay, R.M. 1994. Mutation in the DNA mismatch repair gene
30 homologue *hMLH1* is associated with hereditary non-polyposis colon
cancer. Nature 368:258-261; Fishel, R., Lescoe, M., Rao, M.R.S.,
Copeland, N.J., Jenkins, N.A., Garber, J., Kane, M., and Kolodner, R.

1993. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. *Cell* 7:1027-1038; Leach, F.S., Nicolaides, N.C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L.A., Nystrom-Lahti, M., Guan, X.Y., Zhang, J.,
- 5 Meltzer, P.S., Yu, J.W., Kao, F.T., Chen, D.J., Cerosaletti, K.M., Fournier, R.E.K., Todd, S., Lewis, T., Leach R.J., Naylor, S.L., Weissenbach, J., Mecklin, J.P., Jarvinen, J.A., Petersen, G.M., Hamilton, S.R., Green, J., Jass, J., Watson, P., Lynch, H.T., Trent, J.M., de la Chapelle, A., Kinzler, K.W., and Vogelstein, B. 1993. Mutations of a *mutS* homolog in
- 10 hereditary non-polyposis colorectal cancer. *Cell* 75:1215-1225; Nicolaides, N.C., Papadopoulos, N., Liu, B., Wei, Y.F., Carter, K.C., Ruben, S.M., Rosen, C.A., Haseltine, W.A., Fleischmann, R.D., Fraser, C.M., Adams, M.D., Venter, C.J., Dunlop, M.G., Hamilton, S.R., Petersen, G.M., de la Chapelle, A., Vogelstein, B., and kinzler, K.W. 1994. Mutations of two
- 15 PMS homologs in hereditary nonpolyposis colon cancer. *Nature* 371: 75-80; Nicolaides, N.C., Palombo, F., Kinzler, K.W., Vogelstein, B., and Jiricny, J. 1996. Molecular cloning of the N-terminus of GTBP. *Genomics* 31:395-397; Palombo, F., Hughes, M., Jiricny, J., Truong, O., Hsuan, J. 1994. Mismatch repair and cancer. *Nature* 36:417; Palombo,
- 20 F., Gallinari, P., Iaccarino, I., Lettieri, T., Hughes, M.A., Truong, O., Hsuan, J.J., and Jiricny, J. 1995. GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. *Science* 268:1912-1914; Papadopoulos, N., Nicolaides, N.C., Wei, Y.F., Carter, K.C., Ruben, S.M., Rosen, C.A., Haseltine, W.A., Fleischmann, R.D., Fraser, C.M., Adams,
- 25 M.D., Venter, C.J., Dunlop, M.G., Hamilton, S.R., Petersen, G.M., de la Chapelle, A., Vogelstein, B., and Kinzler, K.W. 1994. Mutation of a *mutL* homolog is associated with hereditary colon cancer. *Science* 263:1625-1629). Germline mutations in four of these genes (*hMSH2*, *hMLH1*, *hPMS1*, and *hPMS2*) have been identified in HNPCC kindred's
- 30 (Bronner, C.E., Baker, S.M., Morrison, P.T., Warren, G., Smith, L.G.,

- Lescoe, M.K., Kane, M., Earabino, C., Lipford, J., Lindblom, A.,
 Tannergard, P., Bollag, R.J., Godwin, A., R., Ward, D.C., Nordenskjold,
 M., Fishel, R., Kolodner, R., and Liskay, R.M. 1994. Mutation in the
 DNA mismatch repair gene homologue *hMLH1* is associated with
 5 hereditary non-polyposis colon cancer. *Nature* 368:258-261; Leach, F.S.,
 Nicolaides, N.C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki,
 P., Sistonen, P., Aaltonen, L.A., Nystrom-Lahti, M., Guan, X.Y., Zhang, J.,
 Meltzer, P.S., Yu, J.W., Kao, F.T., Chen, D.J., Cerosaletti, K.M., Fournier,
 R.E.K., Todd, S., Lewis, T., Leach R.J., Naylor, S.L., Weissenbach, J.,
 10 Mecklin, J.P., Jarvinen, J.A., Petersen, G.M., Hamilton, S.R., Green, J.,
 Jass, J., Watson, P., Lynch, H.T., Trent, J.M., de la Chapelle, A., Kinzler,
 K.W., and Vogelstein, B. 1993. Mutations of a *mutS* homolog in
 hereditary non-polyposis colorectal cancer. *Cell* 75:1215-1225; Liu, B.,
 Parsons, R., Papadopoulos, N., Nicolaides, N.C., Lynch, H.T., Watson, P.,
 15 Jass, J.R., Dunlop, M., Wyllie, A., Peltomaki, P., de la Chapelle, A.,
 Hamilton, S.R., Vogelstein, B., and Kinzler, K.W. 1996. Analysis of
 mismatch repair genes in hereditary non-polyposis colorectal cancer
 patients. *Nat. Med.* 2:169-174; Nicolaides, N.C., Papadopoulos, N., Liu,
 B., Wei, Y.F., Carter, K.C., Ruben, S.M., Rosen, C.A., Haseltine, W.A.,
 20 Fleischmann, R.D., Fraser, C.M., Adams, M.D., Venter, C.J., Dunlop,
 M.G., Hamilton, S.R., Petersen, G.M., de la Chapelle, A., Vogelstein, B.,
 and kinzler, K.W. 1994. Mutations of two PMS homologs in hereditary
 nonpolyposis colon cancer. *Nature* 371: 75-80; Papadopoulos, N.,
 Nicolaides, N.C., Wei, Y.F., Carter, K.C., Ruben, S.M., Rosen, C.A.,
 25 Haseltine, W.A., Fleischmann, R.D., Fraser, C.M., Adams, M.D., Venter,
 C.J., Dunlop, M.G., Hamilton, S.R., Petersen, G.M., de la Chapelle,
 A., Vogelstein, B., and kinzler, K.W. 1994. Mutation of a *mutL* homolog
 is associated with hereditary colon cancer. *Science* 263:1625-1629).
 Though the mutator defect that arises from the MMR deficiency can affect
 30 any DNA sequence, microsatellite sequences are particularly sensitive to

MMR abnormalities (Modrich, P. 1994. Mismatch repair, genetic stability, and cancer. *Science* 266:1959-1960). Microsatellite instability (MI) is therefore a useful indicator of defective MMR. In addition to its occurrence in virtually all tumors arising in HNPCC patients, MI is found in a small fraction of sporadic tumors with distinctive molecular and phenotypic properties (Perucho, M. 1996. Cancer of the microsatellite mutator phenotype. *Biol Chem.* 377:675-684).

HNPCC is inherited in an autosomal dominant fashion, so that the normal cells of affected family members contain one mutant allele of the relevant MMR gene (inherited from an affected parent) and one wild-type allele (inherited from the unaffected parent). During the early stages of tumor development, however, the wild-type allele is inactivated through a somatic mutation, leaving the cell with no functional MMR gene and resulting in a profound defect in MMR activity. Because a somatic mutation in addition to a germ-line mutation is required to generate defective MMR in the tumor cells, this mechanism is generally referred to as one involving two hits, analogous to the biallelic inactivation of tumor suppressor genes that initiate other hereditary cancers (Leach, F.S., Nicolaides, N.C., Papadopoulos, N., Liu, B., Jen, J., Parsons, R., Peltomaki, P., Sistonen, P., Aaltonen, L.A., Nystrom-Lahti, M., Guan, X.Y., Zhang, J., Meltzer, P.S., Yu, J.W., Kao, F.T., Chen, D.J., Cerosaletti, K.M., Fournier, R.E.K., Todd, S., Lewis, T., Leach R.J., Naylor, S.L., Weissenbach, J., Mecklin, J.P., Jarvinen, J.A., Petersen, G.M., Hamilton, S.R., Green, J., Jass, J., Watson, P., Lynch, H.T., Trent, J.M., de la Chapelle, A., Kinzler, K.W., and Vogelstein, B. 1993. Mutations of a *mutS* homolog in hereditary non-polyposis colorectal cancer. *Cell* 75:1215-1225; Liu, B., Parsons, R., Papadopoulos, N., Nicolaides, N.C., Lynch, H.T., Watson, P., Jass, J.R., Dunlop, M., Wyllie, A., Peltomaki, P., de la Chapelle, A., Hamilton, S.R., Vogelstein, B., and Kinzler, K.W. 1996. Analysis of mismatch repair genes in hereditary non-polyposis colorectal cancer

patients. Nat. Med. 2:169-174; Parsons, R., Li, G.M., Longley, M.J., Fang, W.H., Papadopoulos, N., Jen, J., de la Chapelle, A., Kinzler, K.W., Vogelstein, B., and Modrich, P. 1993. Hypermutability and mismatch repair deficiency in RER⁺ tumor cells. Cell 75:1227-1236). In line with
5 this two-hit mechanism, the non-neoplastic cells of HNPCC patients generally retain near normal levels of MMR activity due to the presence of the wild-type allele.

The ability to alter the signal transduction pathways by manipulation of a gene products function, either by over-expression of the
10 wild type protein or a fragment thereof, or by introduction of mutations into specific protein domains of the protein, the so-called dominant-negative inhibitory mutant, were described over a decade in the yeast system *Saccharomyces cerevisiae* by Herskowitz (Nature 329(6136):219-222, 1987). It has been demonstrated that over-expression of wild type gene
15 products can result in a similar, dominant-negative inhibitory phenotype due most likely to the "saturating-out" of a factor, such as a protein, that is present at low levels and necessary for activity; removal of the protein by binding to a high level of its cognate partner results in the same net effect, leading to inactivation of the protein and the associated signal transduction
20 pathway. Recently, work done by Nicolaides et.al. (Nicolaides NC, Littman SJ, Modrich P, Kinzler KW, Vogelstein B 1998. A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype. Mol Cell Biol 18:1635-1641) has demonstrated the utility of introducing dominant negative inhibitory mismatch repair mutants into
25 mammalian cells to confer global DNA hypermutability. The ability to manipulate the MMR process and therefore increase the mutability of the target host genome at will, in this example a mammalian cell, allows for the generation of innovative cell subtypes or variants of the original wild type cells. These variants can be placed under a specified, desired selective
30 process, the result of which is a novel organism that expresses an altered

- biological molecule(s) and has a new trait. The concept of creating and introducing dominant negative alleles of a gene, including the MMR alleles, in bacterial cells has been documented to result in genetically altered prokaryotic mismatch repair genes (Aronshtam A, Marinus MG. 1996. Dominant negative mutator mutations in the mutL gene of *Escherichia coli*. *Nucleic Acids Res* 24:2498-2504; Wu TH, Marinus MG. 1994. Dominant negative mutator mutations in the mutS gene of *Escherichia coli*. *J Bacteriol* 176:5393-400; Brosh RM Jr, Matson SW. 1995. Mutations in motif II of *Escherichia coli* DNA helicase II render the enzyme nonfunctional in both mismatch repair and excision repair with differential effects on the unwinding reaction. *J Bacteriol* 177:5612-5621).
- Furthermore, altered MMR activity has been demonstrated when MMR genes from different species including yeast, mammalian cells, and plants are over-expressed (Fishel, R., Lescoe, M., Rao, M.R.S., Copeland, N.J., Jenkins, N.A., Garber, J., Kane, M., and Kolodner, R. 1993. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. *Cell* 7:1027-1038; Studamire B, Quach T, Alani, E. 1998. *Saccharomyces cerevisiae* Msh2p and Msh6p ATPase activities are both required during mismatch repair. *Mol Cell Biol* 18:7590-7601; Alani E, Sokolsky T, Studamire B, Miret JJ, Lahue RS. 1997. Genetic and biochemical analysis of Msh2p-Msh6p: role of ATP hydrolysis and Msh2p-Msh6p subunit interactions in mismatch base pair recognition. *Mol Cell Biol* 17:2436-2447; Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM, and Collins FS. 2000. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. *Nat. Genet.* 24:27-35).

There is a continuing need in the art for methods of genetically manipulating useful strains of yeast to increase their performance characteristics and abilities.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for rendering yeast cells hypermutable.

It is another object of the invention to provide hypermutable yeast
5 cells.

It is a further object of the invention to provide a method of mutating a gene of interest in a yeast.

It is yet another object of the present invention to provide a method to produce yeast that are hypermutable.

10 It is an object of the invention to provide a method to restore normal mismatch repair activity to hypermutable cells following strain selection.

These and other objects of the invention are provided by one or more of the following embodiments. In one embodiment a method is provided for making a hypermutable yeast. A polynucleotide comprising a
15 dominant negative allele of a mismatch repair gene is introduced into a yeast cell. The cell thus becomes hypermutable.

According to another embodiment a homogeneous composition of cultured, hypermutable yeast cells is provided. The yeast cells comprise a dominant negative allele of a mismatch repair gene.

20 According to still another embodiment of the invention a method is provided for generating a mutation in a gene of interest. A yeast cell culture comprising the gene of interest and a dominant negative allele of a mismatch repair gene is cultivated. The yeast cell is hypermutable. Cells of the culture are tested to determine whether the gene of interest harbors a
25 mutation.

In yet another embodiment of the invention a method is provided for generating a mutation in a gene of interest. A yeast cell comprising the gene of interest and a polynucleotide encoding a dominant negative allele of a mismatch repair gene is grown to create a population of mutated,
30 hypermutable yeast cells. The population of mutated, hypermutable yeast

cells is cultivated under trait selection conditions. Yeast cells which grow under trait selection conditions are tested to determine whether the gene of interest harbors a mutation.

Also provided by the present invention is a method for generating enhanced hypermutable yeast. A yeast cell is exposed to a mutagen. The yeast cell is defective in mismatch repair (MMR) due to the presence of a dominant negative allele of at least one MMR gene. An enhanced rate of mutation of the yeast cell is achieved due to the exposure to the mutagen.

According to still another aspect of the invention a method is provided for generating mismatch repair (MMR)-proficient yeast with new output traits. A yeast cell comprising a gene of interest and a polynucleotide encoding a dominant negative allele of a mismatch repair gene is grown to create a population of mutated, hypermutable yeast cells. The population of mutated, hypermutable yeast cells is cultivated under trait selection conditions. The yeast cells which grow under trait selection conditions are tested to determine whether the gene of interest harbors a mutation. Normal mismatch repair activity is restored to the yeast cells.

These and other embodiments of the invention provide the art with methods that can generate enhanced mutability in yeast as well as providing single-celled eukaryotic organisms harboring potentially useful mutations to generate novel output traits for commercial applications.

DETAILED DESCRIPTION OF THE INVENTION

It is a discovery of the present invention that hypermutable yeast can be made by altering the activity of endogenous mismatch repair activity of host cells. Dominant negative alleles of mismatch repair genes, when introduced and expressed in yeast, increase the rate of spontaneous mutations by reducing the effectiveness of endogenous mismatch repair-mediated DNA repair activity, thereby rendering the yeast highly

susceptible to genetic alterations, *i.e.*, hypermutable. Hypermutable yeast can then be utilized to screen for mutations in a gene or a set of genes in variant siblings that exhibit an output trait(s) not found in the wild-type cells.

5 The process of mismatch repair, also called mismatch proofreading, is an evolutionarily highly conserved process that is carried out by protein complexes described in cells as disparate as prokaryotic cells such as bacteria to more complex mammalian cells (Modrich, P. 1994. Mismatch repair, genetic stability, and cancer. *Science* 266:1959-1960; Parsons, R.,
10 Li, G.M., Longley, M., Modrich, P., Liu, B., Berk, T., Hamilton, S.R., Kinzler, K.W., and Vogelstein, B. 1995. Mismatch repair deficiency in phenotypically normal human cells. *Science* 268:738-740; Perucho, M. 1996. Cancer of the microsatellite mutator phenotype. *Biol Chem.* 377:675-684). A mismatch repair gene is a gene that encodes one of the
15 proteins of such a mismatch repair complex. Although not wanting to be bound by any particular theory of mechanism of action, a mismatch repair complex is believed to detect distortions of the DNA helix resulting from non-complementary pairing of nucleotide bases. The non-complementary base on the newer DNA strand is excised, and the excised base is replaced
20 with the appropriate base that is complementary to the older DNA strand. In this way, cells eliminate many mutations that occur as a result of mistakes in DNA replication, resulting in genetic stability of the sibling cells derived from the parental cell.

 Some wild type alleles as well as dominant negative alleles cause a
25 mismatch repair defective phenotype even in the presence of a wild-type allele in the same cell. An example of a dominant negative allele of a mismatch repair gene is the human gene *hPMS2-134*, which carries a truncation mutation at codon 134 (Parsons, R., Li, G.M., Longley, M., Modrich, P., Liu, B., Berk, T., Hamilton, S.R., Kinzler, K.W., and
30 Vogelstein, B. 1995. Mismatch repair deficiency in phenotypically normal

human cells. Science 268:738-740; Nicolaides NC, Littman SJ, Modrich P, Kinzler KW, Vogelstein B 1998. A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype. Mol Cell Biol 18:1635-1641). The mutation causes the product of this gene to

5 abnormally terminate at the position of the 134th amino acid, resulting in a shortened polypeptide containing the N-terminal 133 amino acids. Such a mutation causes an increase in the rate of mutations, which accumulate in cells after DNA replication. Expression of a dominant negative allele of a mismatch repair gene results in impairment of mismatch repair activity,

10 even in the presence of the wild-type allele. Any mismatch repair allele, which produces such effect, can be used in this invention, whether it is wild-type or altered, whether it derives from mammalian, yeast, fungal, amphibian, insect, plant, or bacteria. In addition, the use of over-expressed wild type MMR gene alleles from human, mouse, plants, and yeast in

15 bacteria has been shown to cause a dominant negative effect on the bacterial hosts MMR activity (Aronshtam A, Marinus MG. 1996. Dominant negative mutator mutations in the mutL gene of *Escherichia coli*. Nucleic Acids Res 24:2498-2504; Wu TH, Marinus MG. 1994. Dominant negative mutator mutations in the mutS gene of *Escherichia coli*. J Bacteriol

20 176:5393-400; Brosh RM Jr, Matson SW. 1995. Mutations in motif II of *Escherichia coli* DNA helicase II render the enzyme nonfunctional in both mismatch repair and excision repair with differential effects on the unwinding reaction. J Bacteriol 177:5612-5621; Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM, and

25 Collins FS. 2000. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. Nat Genet 24:27-35). This suggests that perturbation of the multi-component MMR protein complex can be accomplished by introduction of MMR components from other species into yeast.

Dominant negative alleles of a mismatch repair gene can be obtained from the cells of humans, animals, yeast, bacteria, plants or other organisms. Screening cells for defective mismatch repair activity can identify such alleles. Mismatch repair genes may be mutant or wild type.

- 5 Yeast host MMR may be mutated or not. The term yeast used in this application comprises any organism from the eukaryotic kingdom, including but not limited to *Saccharomyces* sp., *Pichia* sp., *Schizosaccharomyces* sp., *Kluyveromyces* sp., and other fungi (Gellissen, G. and Hollenberg, CP. *Gene* 190(1):87-97, 1997). These organisms can
- 10 be exposed to chemical mutagens or radiation, for example, and can be screened for defective mismatch repair. Genomic DNA, cDNA, mRNA, or protein from any cell encoding a mismatch repair protein can be analyzed for variations from the wild-type sequence. Dominant negative alleles of a mismatch repair gene can also be created artificially, for example, by
- 15 producing variants of the *hPMS2-134* allele or other mismatch repair genes (Nicolaidis NC, Littman SJ, Modrich P, Kinzler KW, Vogelstein B 1998. A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype. *Mol Cell Biol* 18:1635-1641). Various techniques of site-directed mutagenesis can be used. The suitability of such alleles,
- 20 whether natural or artificial, for use in generating hypermutable yeast can be evaluated by testing the mismatch repair activity (using methods described in Nicolaidis NC, Littman SJ, Modrich P, Kinzler KW, Vogelstein B 1998. A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype. *Mol Cell Biol* 18:1635-1641)
- 25 caused by the allele in the presence of one or more wild-type alleles to determine if it is a dominant negative allele.

- A yeast that over-expresses a wild type mismatch repair allele or a dominant negative allele of a mismatch repair gene will become hypermutable. This means that the spontaneous mutation rate of such yeast
- 30 is elevated compared to yeast without such alleles. The degree of elevation

of the spontaneous mutation rate can be at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold that of the normal yeast as measured as a function of yeast doubling/hour.

According to one aspect of the invention, a polynucleotide encoding
5 either a wild type or a dominant negative form of a mismatch repair protein is introduced into yeast. The gene can be any dominant negative allele encoding a protein which is part of a mismatch repair complex, for example, *mutS*, *mutL*, *mutH*, or *mutY* homologs of the bacterial, yeast, plant
10 or mammalian genes (Modrich, P. 1994. Mismatch repair, genetic stability, and cancer. *Science* 266:1959-1960; Prolla, T.A, Pang, Q., Alani, E., Kolodner, R.A., and Liskay, R.M. 1994. MLH1, PMS1, and MSH2 Interaction during the initiation of DNA mismatch repair in yeast. *Science* 264:1091-1093). The dominant negative allele can be naturally occurring or made in the laboratory. The polynucleotide can be in the form of
15 genomic DNA, cDNA, RNA, or a chemically synthesized polynucleotide or polypeptide. The molecule can be introduced into the cell by transformation, electroporation, mating, particle bombardment, or other method described in the literature.

Transformation is used herein as any process whereby a
20 polynucleotide or polypeptide is introduced into a cell. The process of transformation can be carried out in a yeast culture using a suspension of cells. The yeast can be any type classified under the eukaryotic kingdom as by international convention.

In general, transformation will be carried out using a suspension of
25 cells but other methods can also be employed as long as a sufficient fraction of the treated cells incorporate the polynucleotide or polypeptide so as to allow transfected cells to be grown and utilized. The protein product of the polynucleotide may be transiently or stably expressed in the cell. Techniques for transformation are well known to those skilled in the
30 art. Available techniques to introduce a polynucleotide or polypeptide into

a yeast cell include but are not limited to electroporation, viral transduction, cell fusion, the use of spheroplasts or chemically competent cells (e.g., calcium chloride), and packaging of the polynucleotide together with lipid for fusion with the cells of interest. Once a cell has been
5 transformed with the mismatch repair gene or protein, the cell can be propagated and manipulated in either liquid culture or on a solid agar matrix, such as a petri dish. If the transfected cell is stable, the gene will be expressed at a consistent level for many cell generations, and a stable, hypermutable yeast strain results.

10 An isolated yeast cell can be obtained from a yeast culture by chemically selecting strains using antibiotic selection of an expression vector. If the yeast cell is derived from a single cell, it is defined as a clone. Techniques for single-cell cloning of microorganisms such as yeast are well known in the art.

15 A polynucleotide encoding a dominant negative form of a mismatch repair protein can be introduced into the genome of yeast or propagated on an extra-chromosomal plasmid, such as the 2-micron plasmid. Selection of clones harboring a mismatch repair gene expression vector can be accomplished by plating cells on synthetic complete medium lacking the
20 appropriate amino acid or other essential nutrient as described (J. C. Schneider and L. Guarente, *Methods in Enzymology* 194:373,1991). The yeast can be any species for which suitable techniques are available to produce transgenic microorganisms, such as but not limited to genera including *Saccharomyces*, *Schizosaccharomyces*, *Pichia*, *Hansenula*,
25 *Kluyveromyces* and others.

Any method for making transgenic yeast known in the art can be used. According to one process of producing a transgenic microorganism, the polynucleotide is introduced into the yeast by one of the methods well known to those in the art. Next, the yeast culture is grown under conditions
30 that select for cells in which the polynucleotide encoding the mismatch

repair gene is either incorporated into the host genome as a stable entity or propagated on a self-replicating extra-chromosomal plasmid, and the protein encoded by the polynucleotide fragment transcribed and subsequently translated into a functional protein within the cell. Once
5 transgenic yeast is engineered to harbor the expression construct, it is then propagated to generate and sustain a culture of transgenic yeast indefinitely.

Once a stable, transgenic yeast cell has been engineered to express a defective mismatch repair (MMR) protein, the yeast can be cultivated to
10 create novel mutations in one or more target gene(s) of interest harbored within the same yeast cell. A gene of interest can be any gene naturally possessed by the yeast or one introduced into the yeast host by standard recombinant DNA techniques. The target gene(s) may be known prior to the selection or unknown. One advantage of employing such transgenic
15 yeast cells to induce mutations in resident or extra-chromosomal genes within the yeast is that it is unnecessary to expose the cells to mutagenic insult, whether it is chemical or radiation, to produce a series of random gene alterations in the target gene(s). This is due to the highly efficient nature and the spectrum of naturally occurring mutations that result as a
20 consequence of the altered mismatch repair process. However, it is possible to increase the spectrum and frequency of mutations by the concomitant use of either chemical and/or radiation together with MMR defective cells. The net effect of the combination treatment is an increase in mutation rate in the genetically altered yeast that are useful for
25 producing new output traits. The rate of the combination treatment is higher than the rate using only the MMR-defective cells or only the mutagen with wild-type MMR cells.

MMR-defective yeast of the invention can be used in genetic screens for the direct selection of variant sub-clones that exhibit new output
30 traits with commercially desirable applications. This permits one to bypass

the tedious and time consuming steps of gene identification, isolation and characterization.

Mutations can be detected by analyzing the internally and/or externally mutagenized yeast for alterations in its genotype and/or phenotype. Genes that produce altered phenotypes in MMR-defective microbial cells can be discerned by any of a variety of molecular techniques well known to those in the art. For example, the yeast genome can be isolated and a library of restriction fragments of the yeast genome can be cloned into a plasmid vector. The library can be introduced into a “normal” cell and the cells exhibiting the novel phenotype screened. A plasmid can be isolated from those normal cells that exhibit the novel phenotype and the gene(s) characterized by DNA sequence analysis. Alternatively, differential messenger RNA screen can be employed utilizing driver and tester RNA (derived from wild type and novel mutant, respectively) followed by cloning the differential transcripts and characterizing them by standard molecular biology methods well known to those skilled in the art. Furthermore, if the mutant sought is encoded by an extra-chromosomal plasmid, then following co-expression of the dominant negative MMR gene and the gene of interest, and following phenotypic selection, the plasmid can be isolated from mutant clones and analyzed by DNA sequence analysis using methods well known to those in the art. Phenotypic screening for output traits in MMR-defective mutants can be by biochemical activity and/or a readily observable phenotype of the altered gene product. A mutant phenotype can also be detected by identifying alterations in electrophoretic mobility, DNA binding in the case of transcription factors, spectroscopic properties such as IR, CD, X-ray crystallography or high field NMR analysis, or other physical or structural characteristics of a protein encoded by a mutant gene. It is also possible to screen for altered novel function of a protein *in situ*, in isolated form, or in model systems. One can screen for alteration of any property of the yeast

associated with the function of the gene of interest, whether the gene is known prior to the selection or unknown.

The screening and selection methods discussed are meant to illustrate the potential means of obtaining novel mutants with commercially valuable output traits, but they are not meant to limit the many possible ways in which screening and selection can be carried out by those of skill in the art.

Plasmid expression vectors that harbor a mismatch repair (MMR) gene insert can be used in combination with a number of commercially available regulatory sequences to control both the temporal and quantitative biochemical expression level of the dominant negative MMR protein. The regulatory sequences can be comprised of a promoter, enhancer or promoter/enhancer combination and can be inserted either upstream or downstream of the MMR gene to control the expression level. The regulatory sequences can be any of those well known to those in the art, including but not limited to the AOX1, GAP, GAL1, GAL10, PHO5, and PGK promoters harbored on high or low copy number extra-chromosomal expression vectors or on constructs that are integrated into the genome via homologous recombination. These types of regulatory systems have been disclosed in scientific publications and are familiar to those skilled in the art.

Once a microorganism with a novel, desired output trait of interest is created, the activity of the aberrant MMR activity is desirably attenuated or eliminated by any means known in the art. These include but are not limited to removing an inducer from the culture medium that is responsible for promoter activation, curing a plasmid from a transformed yeast cell, and addition of chemicals, such as 5-fluoro-orotic acid to "loop-out" the gene of interest.

In the case of an inducibly controlled dominant negative MMR allele, expression of the dominant negative MMR gene will be turned on

(induced) to generate a population of hypermutable yeast cells with new output traits. Expression of the dominant negative MMR allele can be rapidly turned off to reconstitute a genetically stable strain that displays a new output trait of commercial interest. The resulting yeast strain is now
5 useful as a stable strain that can be applied to various commercial applications, depending upon the selection process placed upon it.

In cases where genetically deficient mismatch repair yeast [strains such as but not limited to: M1 (mutS) and in EC2416 (mutS delta umuDC), and mutL or mutY strains] are used to derive new output traits, transgenic
10 constructs can be used that express wild type mismatch repair genes sufficient to complement the genetic defect and therefore restore mismatch repair activity of the host after trait selection [Grzesiuk, E. et.al. (Mutagenesis 13:127-132, 1998); Bridges, B.A., et.al. (EMBO J. 16:3349-3356, 1997); LeClerc, J.E., Science 15:1208-1211, 1996); Jaworski, A.
15 et.al. (Proc. Natl. Acad. Sci USA 92:11019-11023, 1995)]. The resulting yeast is genetically stable and can be employed for various commercial applications.

The use of over-expression of foreign (exogenous, transgenic) mismatch repair genes from human and yeast such as MSH2, MLH1,
20 MLH3, etc. have been previously demonstrated to produce a dominant negative mutator phenotype in yeast hosts (Shcherbakova, P.V., Hall, M.C., Lewis, M.S., Bennett, S.E., Martin, K.J., Bushel, P.R., Afshari, C.A., and Kunkel, T.A. Mol. Cell Biol. 21(3):940-951; Studamire B, Quach T, Alani, E. 1998. *Saccharomyces cerevisiae* Msh2p and Msh6p ATPase activities
25 are both required during mismatch repair. Mol Cell Biol 18:7590-7601; Alani E, Sokolsky T, Studamire B, Miret JJ, Lahue RS. 1997. Genetic and biochemical analysis of Msh2p-Msh6p: role of ATP hydrolysis and Msh2p-Msh6p subunit interactions in mismatch base pair recognition. Mol Cell Biol 17:2436-2447; Lipkin SM, Wang V, Jacoby R, Banerjee-
30 Basu S, Baxeavanis AD, Lynch HT, Elliott RM, and Collins FS. 2000.

MLH3: a DNA mismatch repair gene associated with mammalian
microsatellite instability. *Nat Genet* 24:27-35). In addition, the use of yeast
strains expressing prokaryotic dominant negative MMR genes as well as
hosts that have genomic defects in endogenous MMR proteins have also
5 been previously shown to result in a dominant negative mutator phenotype
(Evans, E., Sugawara, N., Haber, J.E., and Alani, E. *Mol. Cell.* 5(5):789-
799, 2000; Aronshtam A, Marinus MG. 1996. Dominant negative mutator
mutations in the mutL gene of *Escherichia coli*. *Nucleic Acids Res*
24:2498-2504; Wu TH, Marinus MG. 1994. Dominant negative mutator
10 mutations in the mutS gene of *Escherichia coli*. *J Bacteriol* 176:5393-400;
Brosh RM Jr, Matson SW. 1995. Mutations in motif II of *Escherichia coli*
DNA helicase II render the enzyme nonfunctional in both mismatch repair
and excision repair with differential effects on the unwinding reaction. *J*
Bacteriol 177:5612-5621). However, the findings disclosed here teach the
15 use of MMR genes, including the human PMSR2 gene (Nicolaidis, N.C.,
Carter, K.C., Shell, B.K., Papadopoulos, N., Vogelstein, B., and Kinzler,
K.W. 1995. Genomic organization of the human PMS2 gene family.
Genomics 30:195-206), the related PMS134 truncated MMR gene
(Nicolaidis N.C., Kinzler, K.W., and Vogelstein, B. 1995. Analysis of the
20 5' region of PMS2 reveals heterogenous transcripts and a novel
overlapping gene. *Genomics* 29:329-334), the plant mismatch repair genes
(U.S. patent application S.N. 09/749,601) and those genes that are
homologous to the 134 N-terminal amino acids of the PMS2 gene to create
hypermutable yeast.

25 DNA mutagens can be used in combination with MMR defective
yeast hosts to enhance the hypermutable production of genetic alterations.
This further reduces MMR activity and is useful for generation of
microorganisms with commercially relevant output traits.

The ability to create hypermutable organisms using dominant
30 negative alleles can be used to generate innovative yeast strains that display

new output features useful for a variety of applications, including but not limited to the manufacturing industry, for the generation of new biochemicals, for detoxifying noxious chemicals, either by-products of manufacturing processes or those used as catalysts, as well as helping in

5 remediation of toxins present in the environment, including but not limited to polychlorobenzenes (PCBs), heavy metals and other environmental hazards. Novel yeast strains can be selected for enhanced activity to either produce increased quantity or quality of a protein or non-protein therapeutic molecule by means of biotransformation. Biotransformation is

10 the enzymatic conversion of one chemical intermediate to the next intermediate or product in a pathway or scheme by a microbe or an extract derived from the microbe. There are many examples of biotransformation in use for the commercial manufacturing of important biological and chemical products, including penicillin G, erythromycin, and clavulanic

15 acid. Organisms that are efficient at conversion of "raw" materials to advanced intermediates and/or final products also can perform biotransformation (Berry, A. Trends Biotechnol. 14(7):250-256). The ability to control DNA hypermutability in host yeast strains using a dominant negative MMR (as described above) allows for the generation of

20 variant subtypes that can be selected for new phenotypes of commercial interest, including but not limited to organisms that are toxin-resistant, have the capacity to degrade a toxin *in situ* or the ability to convert a molecule from an intermediate to either an advanced intermediate or a final product. Other applications using dominant negative MMR genes to produce genetic

25 alteration of yeast hosts for new output traits include but are not limited to recombinant production strains that produce higher quantities of a recombinant polypeptide as well as the use of altered endogenous genes that can transform chemical or catalyze manufacturing downstream processes. A regulatable dominant negative MMR phenotype can be used

30 to produce a yeast strain with a commercially beneficial output trait. Using

this process, single-celled yeast cells expressing a dominant negative MMR can be directly selected for the phenotype of interest. Once a selected yeast with a specified output trait is isolated, the hypermutable activity of the dominant negative MMR allele can be turned-off by several methods well known to those skilled in the art. For example, if the dominant-negative allele is expressed by an inducible promoter system, the inducer can be removed or depleted. Such systems include but are not limited to promoters such as: lactose inducible GAL₁-GAL10 promoter (M. Johnston and R.W. Davis, *Mol. Cell Biol.* 4:1440, 1984); the phosphate inducible PHO5 promoter (A. Miyanohara, A. Toh-e, C. Nosaki, F. Nosaki, F. Hamada, N. Ohtomo, and K. Matsubara. *Proc. Natl. Acad. Sci. U.S.A.* 80:1, 1983); the alcohol dehydrogenase I (ADH) and 3-phosphoglycerate kinase (PGK) promoters, that are considered to be constitutive but can be repressed/de-repressed when yeast cells are grown in non-fermentable carbon sources such as but not limited to lactate (G. Ammerer, *Methods in Enzymology* 194:192, 1991; J. Mellor, M.J. Dobson, N.A. Roberts, M.F. Tuite, J.S. Emtage, S. White, D.A. Lowe, T. Patel, A.J. Kingsman, and S.M. Kingsman, *Gene* 24:563, 1982); S. Hahn and L. Guarente, *Science* 240:317, 1988); Alcohol oxidase (AOX) in *Pichia pastoris* (Tschopp, JF, Brust, PF, Cregg, JM, Stillman, CA, and Gingeras, TR. *Nucleic Acids Res.* 15(9):3859-76, 1987; and the thiamine repressible expression promoter nmt1 in *Schizosaccharomyces pombe* (Moreno, MB, Duran, A., and Ribas, JC. *Yeast* 16(9):861-72, 2000). Yeast cells can be transformed by any means known to those skilled in the art, including chemical transformation with LiCl (Mount, R.C., Jordan, B.E., and Hadfield, C. *Methods Mol. Biol.* 53:139-145, 1996) and electroporation (Thompson, JR, Register, E., Curotto, J., Kurtz, M. and Kelly, R. *Yeast* 14(6):565-71, 1998). Yeast cells that have been transformed with DNA can be selected for growth by a variety of methods, including but not restricted to selectable markers (URA3; Rose, M., Grisafi, P., and Botstein, D. *Gene* 29:113, 1984; LEU2;

A. Andreadis, Y., Hsu, M., Hermodson, G., Kohlhaw, and P. Schimmel. J. Biol. Chem. 259:8059,1984; ARG4; G. Tschumper and J. Carbon. Gene 10:157, 1980; and HIS3; K. Struhl, D.T. Stinchcomb, S., Scherer, and R.W. Davis Proc. Natl. Acad. Sci. U.S.A. 76:1035,1979) and drugs that inhibit
5 growth of yeast cells (tunicamycin, TUN; S. Hahn, J., Pinkham, R. Wei, R., Miller, and L. Guarente. Mol. Cell Biol. 8:655,1988). Recombinant DNA can be introduced into yeast as described above and the yeast vectors can be harbored within the yeast cell either extra-chromosomally or integrated into a specific locus. Extra-chromosomal based yeast expression vectors
10 can be either high copy based (such as the 2- μ m vector Yep13; A.B. Rose and J.R. Broach, Methods in Enzymology 185:234,1991), low copy centromeric vectors that contain autonomously replicating sequences (ARS) such as YRp7 (M. Fitzgerald-Hayes, L. Clarke, and J. Carbon, Cell 29:235,1982) and well as integration vectors that permit the gene of interest
15 to be introduced into specified locus within the host genome and propagated in a stable manner (R.J. Rothstein, Methods in Enzymology 101:202, 1991). Ectopic expression of MMR genes in yeast can be attenuated or completely eliminated at will by a variety of methods, including but not limited to removal from the medium of the specific
20 chemical inducer (e.g deplete galactose that drives expression of the GAL10 promoter in *Saccharomyces cerevisiae* or methanol that drives expression of the AOX1 promoter in *Pichia pastoris*), extra-chromosomally replicating plasmids can be "cured" of expression plasmid by growth of cells under non-selective conditions (e.g. YEp13 harboring
25 cells can be propagated in the presence of leucine,) and cells that have genes inserted into the genome can be grown with chemicals that force the inserted locus to "loop-out" (e.g., integrants that have URA3 can be selected for loss of the inserted gene by growth of integrants on 5-fluoro-
30 197:345-346,1984). Whether by withdrawal of inducer or treatment of

yeast cells with chemicals, removal of MMR expression results in the re-establishment of a genetically stable yeast cell-line. Thereafter, the lack of mutant MMR allows the endogenous, wild type MMR activity in the host cell to function normally to repair DNA. The newly generated mutant

5 yeast strains that exhibit novel, selected output traits are suitable for a wide range of commercial processes or for gene/protein discovery to identify new biomolecules that are involved in generating a particular output trait. While it has been documented that MMR deficiency can lead to as much as a 1000-fold increase in the endogenous DNA mutation rate of a host, there

10 is no assurance that MMR deficiency alone will be sufficient to alter every gene within the DNA of the host bacterium to create altered biochemicals with new activity(s). Therefore, the use of chemical mutagens and their respective analogues such as ethidium bromide, EMS, MNNG, MNU, Tamoxifen, 8-Hydroxyguanine, as well as others such as those taught in:

15 Khromov-Borisov, N.N., et.al. (Mutat. Res. 430:55-74, 1999); Ohe, T., et.al. (Mutat. Res. 429:189-199, 1999); Hour, T.C. et.al. (Food Chem. Toxicol. 37:569-579, 1999); Hrelia, P., et.al. (Chem. Biol. Interact. 118:99-111, 1999); Garganta, F., et.al. (Environ. Mol. Mutagen. 33:75-85, 1999); Ukawa-Ishikawa S., et.al. (Mutat. Res. 412:99-107, 1998);

20 www.ehs.utah.edu/ohh/mutagens, etc. can be used to further enhance the spectrum of mutations and increase the likelihood of obtaining alterations in one or more genes that can in turn generate host yeast with a desired new output trait(s). Mismatch repair deficiency leads to hosts with an increased resistance to toxicity by chemicals with DNA damaging activity. This

25 feature allows for the creation of additional genetically diverse hosts when mismatch defective yeast are exposed to such agents, which would be otherwise impossible due to the toxic effects of such chemical mutagens [Colella, G., et.al. (Br. J. Cancer 80:338-343, 1999); Moreland, N.J., et.al. (Cancer Res. 59:2102-2106, 1999); Humbert, O., et.al. (Carcinogenesis 20:205-214, 1999); Glaab, W.E., et.al. (Mutat. Res. 398:197-207, 1998)].

30

Moreover, mismatch repair is responsible for repairing chemically-induced DNA adducts, therefore blocking this process could theoretically increase the number, types, mutation rate and genomic alterations of a yeast [Rasmussen, L.J. et.al. (Carcinogenesis 17:2085-2088, 1996); Sledziowska-Gojska, E., et.al. (Mutat. Res. 383:31-37, 1997); and Janion, C. et.al. (Mutat. Res. 210:15-22, 1989)]. In addition to the chemicals listed above, other types of DNA mutagens include ionizing radiation and UV-irradiation, which is known to cause DNA mutagenesis in yeast, can also be used to potentially enhance this process (Lee CC, Lin HK, Lin JK. 1994. A reverse mutagenicity assay for alkylating agents based on a point mutation in the beta-lactamase gene at the active site serine codon. Mutagenesis 9:401-405; Vidal A, Abril N, Pueyo C. 1995. DNA repair by Ogt alkyltransferase influences EMS mutational specificity. Carcinogenesis 16:817-821). These agents, which are extremely toxic to host cells and therefore result in a decrease in the actual pool size of altered yeast cells are more tolerated in MMR defective hosts and in turn permit an enriched spectrum and degree of genomic mutagenesis.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples that will be provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLES

5 **Example 1: Generation of inducible MMR dominant negative allele vectors and yeast cells harboring the expression vectors**

Yeast expression constructs were prepared to determine if the human PMS2 related gene (hPMSR2) (Nicolaidis et al. Genomics 30(2):195-206) and the human PMS134 gene (Nicolaidis NC, Littman SJ, 10 Modrich P, Kinzler KW, Vogelstein B 1998. A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype. Mol Cell Biol 18:1635-1641) are capable of inactivating the yeast MMR activity and thereby increase the overall frequency of genomic hypermutation, a consequence of which is the generation of variant sib cells with novel 15 output traits following host selection. For these studies, a plasmid encoding the hPMS134 cDNA was altered by polymerase chain reaction (PCR). The 5' oligonucleotide has the following structure: 5'-ACG CAT ATG GAG CGA GCT GAG AGC TCG AGT-3' that includes the NdeI restriction site CAT ATG. The 3'-oligonucleotide has the following structure: 5'-GAA 20 TTC TTA TCA CGT AGA ATC GAG ACC GAG GAG AGG GTT AGG GAT AGG CTT ACC AGT TCC AAC CTT CGC CGA TGC-3' that includes an EcoRI site GAA TTC and the 14 amino acid epitope for the V5 antibody. The oligonucleotides were used for PCR under standard conditions that included 25 cycles of PCR (95°C for 1 minute, 55°C for 1 25 minute, 72°C for 1.5 minutes for 25 cycles followed by 3 minutes at 72°C). The PCR fragment was purified by gel electrophoresis and cloned into pTA2.1 (Invitrogen) by standard cloning methods (Sambrook et al., Molecular Cloning: A Laboratory Manual, Third Edition, 2001), creating the plasmid pTA2.1-hPMS134. pTA2.1-hPMS134 was digested with the 30 restriction enzyme EcoRI to release the insert which was cloned into EcoRI

restriction site of pPIC3.5K (Invitrogen). The following strategy, similar to that described above to clone human PMS134, was used to construct an expression vector for the human related gene PMSR2. First, the hPMSR2 fragment was amplified by PCR to introduce two restriction sites, an NdeI restriction site at the 5'- end and an Eco RI site at the 3'-end of the fragment. The 5'-oligonucleotide that was used for PCR has the following structure: 5'-ACG CAT ATG TGT CCT TGG CGG CCT AGA-3' that includes the NdeI restriction site CAT ATG. The 3'-oligonucleotide used for PCR has the following structure: 5'-GAA TTC TTA TTA CGT AGA ATC GAG ACC GAG GAG AGG GTT AGG GAT AGG CTT ACC CAT GTG TGA TGT TTC AGA GCT-3' that includes an EcoRI site GAA TTC and the V5 epitope to allow for antibody detection. The plasmid that contained human PMSR3 in pBluescript SK (Nicolaidis et al. Genomics 30 (2):195-206,1995) was used as the PCR target with the hPMS2-specific oligonucleotides above. Following 25 cycles of PCR (95°C for 1 minute, 55°C for 1 minute, 72°C for 1.5 minutes for 25 cycles followed by 3 minutes at 72°C). The PCR fragment was purified by gel electrophoresis and cloned into pTA2.1 (Invitrogen) by standard cloning methods (Sambrook et al., Molecular Cloning: A Laboratory Manual, Third Edition, 2001), creating the plasmid pTA2.1-hR2. pTA2.1-hR2 was next digested with the restriction enzyme EcoRI to release the insert (there are two EcoRI restriction sites in the multiple cloning site of pTA2.1 that flank the insert) and the inserted into the yeast expression vector pPIC3.5K (Invitrogen).

Pichia pastoris yeast cells were transformed with pPIC3.5K vector, pPIC3.5K-pms134, and pPIC3.5K-hR2 as follows. First, 5ml of YPD (1% yeast extract, 2% bacto-peptone, 1% dextrose) medium was inoculated with a single colony from a YPD plate (same as YPD liquid but add 2% difco-agar to plate) and incubated with shaking overnight at 30°C. The overnight culture was used to inoculate 500ml of YPD medium (200ul of overnight culture) and the culture incubated at 30°C until the optical density at 600nm

reached 1.3 to 1.5. The cells were then spun down (4000 x g for 10 minutes), and then washed 2 times in sterile water (one volume each time), then the cells suspended in 20ml of 1M sorbitol. The sorbitol/cell suspension was spun down (4,000xg for 10 minutes) and suspended in 1ml
5 of 1M sorbitol. 80ul of the cell suspension was mixed with 5 to 10ug of linearized plasmid DNA and placed in a 0.2cm cuvette, pulsed length 5 to 10milliseconds at field strength of 7,500V/cm. Next, the cells are diluted in 1ml of 1M sorbitol and transferred to a 15ml tube and incubated at 30°C for 1 to 2 hours without shaking. Next, the cells are spun out (4,000 x G
10 for 10 minutes) and suspended in 100ul of sterile water, and 50ul/plate spread onto the appropriate selective medium plate. The plates are incubated for 2 to 3 days at 30°C and colonies patched out onto YPD plates for further testing.

15 Example 2: Generation of hypermutable yeast with inducible dominant negative alleles of mismatch repair genes

Yeast clones expressing human PMS2 homologue PMS-R2 or empty vector were grown in BMG (100mM potassium phosphate, pH
20 6.0, 1.34% YNB (yeast nitrogen base), 4×10^{-5} % biotin, 1% glycerol) liquid culture for 24 hr at 30°C. The next day, cultures were diluted 1:100 in MM medium (1.34% YNB, 4×10^{-5} % biotin, 0.5% methanol) and incubated at 30°C with shaking. Cells were removed for mutant selection at 24 and 48 hours post methanol
25 induction as described below (see **EXAMPLE 3**).

EXAMPLE 3: Dominant negative MMR genes can produce new genetic variants and commercially viable output traits in yeast.

The ability to express MMR genes in yeast, as presented in example 2, demonstrate the ability to generate genetic alterations and new phenotypes in yeast expressing dominant negative MMR genes. In this **example** we teach the utility of this method to create eukaryotic strains with commercially relevant output traits.

5

GENERATION OF URACIL DEPENDENT YEAST STRAIN

One example of utility is the generation of a yeast strain that is mutant for a particular metabolic product, such as an amino acid or nucleotide. Engineering such a yeast strain will allow for recombinant manipulation of the yeast strain for the introduction of genes for scalable process of recombinant manufacturing. In order to demonstrate that MMR can be manipulated in yeast to generate mutants that lack the ability to produce specific molecular building blocks, the following experiment was performed. Yeast cells that express a methanol inducible human PMS2 homologue, hPMS2-R2 (as described in example 1 above), were grown in BMY medium overnight then diluted 1:100 and transferred to MM medium, which results in activation of the AOX promoter and production of the hPMS2-R2 MMR gene that is resident within the yeast cell. Control cells were treated the same manner; these cells contain the pPIC3.5 vector in yeast and lack an insert. Cells were induced for 24 and 48 hours and then selected for uracil requiring mutations as follows. The cells were plated to 5-FOA medium (Boeke, J.D., LaCroute, F., and Fink, G.R. Mol. Gen. Genet. 197:345-345,1984). The plates are made as follows: (2X concentrate (filter sterilize): yeast nitrogen base 7 grams; 5-fluoro-orotic acid 1 gram; uracil 50 milligrams; glucose 20 grams; water to 500 ml; Add to 500 ml 4% agar (autoclaved) and pour plates. Cells are plated on 5-FOA plates (0, 24 and 48 hour time points) and incubated at 30°C for between 3 and 5 days. Data from a typical experiment is shown in Table 1. No uracil requiring clones were observed in the un-induced or induced culture in yeast cells that harbor the "empty" vector whereas those cells that harbor the MMR gene hPMS2-R2 have clones that are capable of growth on the selection medium. Note that the un-induced culture of hPMS2-R2 does not have any colonies that are resistant to 5-FOA,

demonstrating that the gene must be induced for the novel phenotype to be generated. It has been demonstrated that the mutagens (such as ethyl methyl sulfonate result in a low number of ura⁻ mutants and that the spontaneous mutation rate for generating this class of mutants is low (Boeke, J.D., LaCrute, F. and Fink, G.R. Mol. Gen. Genet. 197:345-346,1984).

Table 1: Generation of uracil requiring mutant *Pichia pastoris* yeast cells. #

Represents at 24 hour methanol induction and @ a 48 hour induction. For comparison a wild type yeast cell treated/un-treated is shown (Galli, A. and Schiestl, R.H. Mutat.

Res. 429(1):13-26,1999).

Strain	Seeded	ura ⁻	URA ⁺	Frequenc y (ura ⁻ cells)
Wt	100,000	0	~100,000	0
Empty	100,000	0	~100,000	0
pMOR ^{ye-1#}	100,000	14	~100,000	1/7,142
pMOR ^{ye2@}	100,000	123	~100,000	1/813
Wt	100,000	1-0.1	100,000	1/10 ^{5-6*}
Mutagen	100,000	10	100,000	1/10,000

GENERATION OF HEAT-RESISTANT PRODUCER STRAINS

One example of commercial utility is the generation of heat-resistant recombinant protein producer strains. In the scalable process of recombinant manufacturing, large-scale fermentation of both prokaryotes and eukaryotes results in the generation of excessive heat within the culture. This heat must be dissipated by physical means such as using cooling jackets that surround the culture while it is actively growing and producing product. Production of a yeast strain that can resist high temperature growth effectively would be advantageous for large-scale recombinant manufacturing processes. To this end, the yeast strain as described in

EXAMPLE 2 can be grown in the presence of methanol to induce the dominant negative MMR gene and the cells grown for various times (e.g. 12, 24, 36 and 48 hours) then put on plates and incubated at elevated temperatures to select for mutants that resist high temperature growth (e.g. 37°C or 42°C). These strains would be useful for fermentation development and scale-up of processes and should result in a decrease in manufacturing costs due to the need to cool the fermentation less often.

GENERATION OF HIGH RECOMBINANT PROTEIN PRODUCER STRAINS AND STRAINS WITH LESS ENDOGENOUS PROTEASE ACTIVITY

10

Yeast is a valuable recombinant-manufacturing organism since it is a single celled organism that is inexpensive to grow and easily lends itself to fermentation at scale. Further more, many eukaryotic proteins that are incapable of folding effectively when expressed in *Escherichia coli* systems fold with the proper conformation in yeast and are structurally identical to their mammalian counterparts. There are several inherent limitations of many proteins that are expressed in yeast including over and/or inappropriate glycosylation of the recombinant protein, proteolysis by endogenous yeast enzymes and insufficient secretion of recombinant protein from the inside of the yeast cell to the medium (which facilitates purification). To generate yeast cells that with this ability to over-secrete proteins, or with less endogenous protease activity and or less hyper-glycosylation activity yeast cells as described in example 1 can be grown with methanol for 12, 24, 36 and 48 hours and yeast cells selected for the ability to over-secrete the protein or interest, under-glycosylate it or a cell with attenuated or no protease activity. Such a strain will be useful for recombinant manufacturing or other commercial purposes and can be combined with the heat resistant strain outlined above. For example, a mutant yeast cell that is resistant to high temperature growth and can secrete large amounts of protein into the medium would result.

Similar results were observed with other dominant negative mutants such as the PMSR2, PMSR3, and the human MLH1 proteins.

EXAMPLE 4: Mutations generated in the host genome of yeast by defective MMR are genetically stable

5

As described in example 3 manipulation of the MMR pathway in yeast results in alterations within the host genome and the ability to select for a novel output traits, for example the ability of a yeast cell to require a specific nutrient. It is important that the mutations introduced by the MMR pathway is genetically stable and passed to daughter cells reproducibly once the wild type MMR pathway is re-established. To determine the genetic stability of mutations introduced into the yeast genome the following experiment was performed. Five independent colonies from pPIC3.5K-hPMS2-R2 that are ura^- , five wild type control cells (URA^+) and five pPIC3.5K transformed cells ("empty vector") were grown overnight from an isolated colony in 5 ml of YPD (1% yeast extract, 2% bacto-peptone and 1% dextrose) at 30°C with shaking. The YPD medium contains all the nutrients necessary for yeast to grow, including uracil. Next, 1 μ L of the overnight culture, which was at an optical density (OD) as measured at 600nm of >3.0, was diluted to an OD₆₀₀ of 0.01 in YPD and the culture incubated with shaking at 30°C for an additional 24 hours. This process was repeated 3 more times for a total of 5 overnight incubations. This is the equivalent of greater than 100 generations of doublings (from the initial colony on the plate to the end of the last overnight incubation. Cells (five independent colonies that are ura^- and five that were wild type were then plated onto YPD plates at a cell density of 300 to 1,000 cells/plate and incubated for two days at 30°C. The cells from these plates were replica plated to the following plates and scored for growth following three days incubation at 30°C; Synthetic Complete (SC) SC-ura (1.34% yeast nitrogen base and ammonium sulfate; 4 x 10⁻⁵ % biotin; supplemented with all amino acids, NO supplemental uracil; 2% dextrose and 2% agar); SC +URA (same as SC-ura but supplement plate with 50 mg uracil/liter medium), and YPD plates. They were replica plated in the following order- SC -ura, SC complete, YPD. If the novel output trait

10
15
20
25
30

that is resident within the yeast genome that was generated by expression of the mutant MMR (in this example the human homologue of PMS2, hPMS2-R2) is unstable, the uracil dependent cells should "revert" back a uracil independent phenotype. If the phenotype is stable, growth of the mutant cells under non-selective conditions should result in yeast cells that maintain their viability dependence on exogenous supplementation with uracil. As can be seen in the data presented in Table 2, the uracil dependent phenotype is stable when the yeast cells are grown under non-selective conditions, demonstrating that the MMR-generated phenotype derived from mutation in one of the uracil biosynthetic pathway genes is stable genetically.

Strain	Seeded	-ura	+URA	YPD
Wt	650	650	650	650
Empty	560	560	560	560
pMOR ^{ye-1#}	730	0	730	730

These data demonstrate the utility of employing an inducible expression system and a dominant negative MMR gene in a eukaryotic system to generate genetically altered strains. The strain developed in this example, a yeast strain that now requires addition of uracil for growth, is potentially useful as a strain for recombinant manufacturing; by constructing an expression vector that harbors the wild type URA3 gene on either an integration plasmid or an extra-chromosomal vector it is now possible to transform and create novel cells expressing the a protein of interest. It is also possible to modify other resident genes in yeast cells and select for mutations in genes that that give other useful phenotypes, such as the ability to carry out a novel bio-transformation. Furthermore, it is possible to express a gene extra-chromosomally in a yeast cell that has altered MMR activity as described above and select for mutations in the extra-chromosomal gene. Therefore, in a similar manner to that described above the mutant yeast cell can be put under specific selective pressure and a novel protein with commercially important biochemical attributes selected. These examples are meant only as illustrations and are not meant to limit the scope of the present invention.

Finally, as described above once a mutation has been introduced into the gene of interest the MMR activity is attenuated or completely abolished. The result is a yeast cell that harbors a stable mutation in the target gene(s) of interest.

5 **EXAMPLE 5: Enhanced Generation of MMR-Defective Yeast and Chemical Mutagens for the Generation of New Output Traits**

It has been previously documented that MMR deficiency yields to increased mutation frequency and increased resistance to toxic effects of chemical mutagens (CM) and their
10 respective analogues such as but not limited to those as: ethidium bromide, EMS, MNNG, MNU, Tamoxifen, 8-Hydroxyguanine, as well as others listed but not limited to in publications by: Khromov-Borisov, N.N., et.al. *Mutat. Res.* 430:55-74, 1999; Ohe, T., et.al. (*Mutat. Res.* 429:189-199, 1999; Hour, T.C. et.al. *Food Chem. Toxicol.* 37:569-579, 1999; Hrelia, P., et.al. *Chem. Biol. Interact.* 118:99-111, 1999; Garganta, F., et.al. *Environ. Mol.*
15 *Mutagen.* 33:75-85, 1999; Ukawa-Ishikawa S., et.al. *Mutat. Res.* 412:99-107, 1998; www.ehs.utah.edu/ohh/mutagens; Marcelino LA, Andre PC, Khrapko K, Collier HA, Griffith J, Thilly WG. Chemically induced mutations in mitochondrial DNA of human cells: mutational spectrum of N-methyl-N'-nitro-N-nitrosoguanidine. *Cancer Res* 1998 Jul 1;58(13):2857-62; Koi M, Umar A, Chauhan DP, Cherian SP, Carethers JM, Kunkel TA,
20 Boland CR. Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces N-methyl-N'-nitro-N-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. *Can res* 1994 54:4308-4312, 1994. Mismatch repair provokes chromosome aberrations in hamster cells treated with methylating agents or 6-thioguanine, but not with ethylating agents. To demonstrate the ability of CMs to increase the
25 mutation frequency in MMR defective yeast cells, we would predict that exposure of yeast cells to CMs in the presence or absence of methanol (which induces the expression of the resident human homologue to PMS2, hPMS2-R2) will result in an augmentation of mutations within the yeast cell.

Yeast cells that express hPMS2-R2 (induced or un-induced) and empty vector control
30 cells are grown as described in examples 2 and 3) and for 24 hours and diluted into MM

medium as described above. Next, the cells in MM are incubated either with or without increasing amounts of ethyl methane sulfonate (EMS) from 0, 1, 10, 50, 100, and 200 μ M. 10 μ L aliquots of culture (diluted in 300 μ L MM) and incubated for 30 minutes, 60 minutes, and 120 minutes followed by plating cells onto 5-FOA plates as described in example 3
5 above. Mutants are selected and scored as above. We would predict that there will be an increase in the frequency of *ura⁻* mutants in the PMS2-R2 cultures that are induced with methanol as compared to the uninduced parental or wild type strain. In a further extension of this example, human PMS2-R2 harboring cells will be induced for 24 and 48 hours then mutagenized with EMS. This will allow the MMR gene to be fully active and expressed at
10 high levels, thereby resulting in an increase in the number of *ura⁻* mutants obtained. We would predict that there will be no change in the number of *ura⁻* mutants obtained in the uninduced parental control or the wild type "empty vector" cells.

This example demonstrates the use of employing a regulated dominant negative
15 MMR system plus chemical mutagens to produce enhanced numbers of genetically altered yeast strains that can be selected for new output traits. This method is useful for generating such organisms for commercial applications such as but not limited to recombinant manufacturing, biotransformation, and altered biochemicals with enhanced activities. It is also useful to obtain alterations of protein activity from ectopically expressed proteins
20 harbored on extra-chromosomal expression vectors similar to those described in example 4 above.

EXAMPLE 6: Alternative Methods to Inhibition of Yeast MMR Activity

The inhibition of MMR activity in a host organism can be achieved by introducing a
25 dominant negative allele as shown in the examples above. This application also teaches us the use of using regulated systems to control MMR in yeast to generate genetic diversity and output traits for commercial applications. Additional methods to regulate the suppression of MMR activity of a host are by using genetic recombination to knock out alleles of a MMR gene within the cell of interest. This can be accomplished by use of homologous
30 recombination that disrupts the endogenous MMR gene; 2) blocking MMR protein

dimerization with other subunits (which is required for activity) by the introduction of polypeptides or antibodies into the host via transfection methods routinely used by those skilled in the art (e.g. electroporation); or 3) decreasing the expression of a MMR gene using anti-sense oligonucleotides.

5

MMR gene knockouts. We intend to generate disrupted targeting vectors of a particular MMR gene and introduce it into the genome of yeast using methods standard in the art. Yeast exhibiting hypermutability will be useful to produce genetically diverse offspring for commercial applications. Yeast will be confirmed to have lost the expression of the MMR gene using standard northern and biochemical techniques (as described in reference 31).

10

MMR gene loci can be knocked out, strains selected for new output traits and MMR restored by introducing a wild type MMR gene to complement the KO locus. Other strategies include using KO vectors that can target a MMR gene locus, select for host output traits and then have the KO vector "spliced" from the genome after strain generation.

15

Blocking peptides. MMR subunits (MutS and MutL proteins) interact to form active MMR complexes. Peptides are able to specifically inhibit the binding of two proteins by competitive inhibition. Introduction into cells of peptides or antibodies to conserved domains of a particular MMR gene to disrupt activity is straightforward to those skilled in the art.

20

Yeast will be verified for loss of expression of the MMR activity by standard northern and/or biochemical techniques (as described in Nicolaides NC, Littman SJ, Modrich P, Kinzler KW, Vogelstein B 1998. A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype. Mol Cell Biol 18:1635-1641). Yeast exhibiting hypermutability will be useful to produce genetically diverse sibs for commercial applications.

25

Discussion

The results described above will lead to several conclusions. First, expression of dominant negative MMR proteins results in an increase in microsatellite instability and hypermutability in yeast. The hypermutability of the yeast cell is due to the inhibition of the resident, endogenous MMR biochemical activity in these hosts. This method provides a claim

30

for use of MMR genes and their encoded products for the creation of hypermutable yeast to produce new output traits for commercial applications.

EXAMPLES OF MMR GENES AND ENCODED POLYPEPTIDES

5 Yeast MLH1 cDNA (accession number U07187)

```

1 aaataggaat gtgatacctt ctattgcatg caaagatagt gtaggaggcg ctgctattgc
61 caaagacttt tgagaccgct tgctgtttca ttatagttga ggagttctcg aagacgagaa
10 121 attagcagtt ttcggtgttt agtaatcgcg ctagcatgct aggacaattt aactgcaaaa
181 ttttgatacg atagtgatag taaatggaag gtaaaaataa catagaccta tcaataagca
241 atgtctctca gaataaaagc acttgatgca tcagtgggta acaaaattgc tgcaggtgag
301 atcataatat cccccgtaaa tgctctcaaa gaaatgatgg agaattccat cgatgcgaat
361 gctacaatga ttgatattct agtcaaggaa ggaggaatta aggtacttca aataacagat
421 aacggatctg gaattaataa agcagacctg ccaatcttat gtgagcgatt cagcagctcc
15 481 aaattacaaa aattcgaaga tttgagtcag attcaaactg atggattccg aggagaagct
541 ttagccagta tctcacatgt ggcaagagtc acagtaacga caaaagttaa agaagacaga
601 tgtgcatgga gagtttcata tgcagaagggt aagatgtttg aaagccccaac acctgtgtgt
661 ggaaaagacg gtaccacgat cctagttgaa gacctttttt tcaatatctc tctagatta
721 agggccttga ggtcccataa tgatgaattac tctaaaatat tagatgttgt cggcgataac
20 781 gccattcatt ccaaggacat tggtttttct tgtaaaaagt tgcggagactc taattattct
841 ttatcagtta aaccttcata tacagtcacg gataggatta ggactgtgtt caataaatct
901 gtggcttcga atttaattac ttttcatatc agcaaagtag aagatttaaa cctggaaagc
961 gttgatggaa aggtgtgtaa tttgaatttc atatccaaaa agtccatttc attaattttt
1021 ttcattaata atagactagt gacatgtgat cttctaagaa gagctttgaa cagcgtttac
25 1081 tccaattatc tgccaaaggg ctccagacct tttattttat tgggaattgt tatagatccg
1141 gcggctgttg atgttaacgt tcacccgaca aagagagagg ttcgtttcct gagccaagat
1201 gagatcatag agaaaatcgc caatcaattg cagccgaat tatctgccat tgatacttca
1261 cgtactttca aggttcttc aatttcaaca aacaagccag agtcattgat accatttaat
30 1321 gacaccatag aaagtgatag gaataggaag agtctccgac aagcccaagt ggtagagaat
1381 tcatatacga cagccaatag tcaactaagg aaagcgaaaa gacaagagaa taaactagtc
1441 agaatagatg cttcacaagc taaaattacg tcatttttat cctcaagtca acagttcaac
1501 tttgaaggat cgtctacaaa gcgacaactg agtgaacca agtgaacca aaccgcgtga tgccaataca
1561 tcccaagagg cagaaaagct gacactaaat gaaagcgaac aaccgcgtga tgccaataca
35 1621 atcaatgata atgacttgaa ggatcaacct aagaagaaac aaaagttggg ggattataaa
1681 gttccaagca ttgccgatga cgaaaagaat gcactccga tttcaaaaaga cgggtatatt
1741 agagtaccta aggagcgagt taatgttaat cttacgagta tcaagaaatt gcgtgaaaaa
1801 gtatagattt cgatacatcg agaactaaca gacatttttg caaatttgaa ttacgttggg
1861 gttgtagatg aggaagaag attagccgct attcagcatg acttaaagct ttttttaata
40 1921 gattacggat ctgtgtgcta tgagctattc tatcagattg gtttgacaga cttcgcaaac
1981 tttggttaaga taaacctaca gagtacaaat gtgtcagatg atatagtttt gtataatctc
2041 ctatcagaat ttgacgagtt aaatgacgat gcttccaaag aaaaaataat tagtaaaata
2101 tgggacatga gcagtatgct aaatgagtac tattccatag aattggtgaa tgatggtcta
2161 gataatgact taaagtctgt gaagctaaaa tctctaccac tacttttaaa aggctacatt
2221 ccattctctg tcaagttacc attttttata tatcgccctg gtaaagaagt tgattgggag
45 2281 gatgaacaag agtgtctaga tggattttta agagagattg cattactcta tatacctgat
2341 atggttccga aagtcgatac actcgatgca tcgttgtcag aagacgaaaa agcccagttt
2401 ataaatagaa aggaacacat atcctcatta ctagaacacg ttctcttccc ttgtatcaaa
2461 cgaaggttcc tggcccctag acacattctc aaggatgtcg tggaaatagc caaccttcca
50 2521 gatctataca aagtttttga gaggtgttaa ctttaaaacg ttttggctgt aataccaaaag
2581 tttttgttta tttcctgagt gtgattgtgt ttcatgtgaa agtgtatgcc ctttcttta
2641 acgattcatc cgcgagattt caaaggatat gaaatatggt tgcagttagg aaagtatgtc
2701 agaaatgtat attcggattg aaactcttct aatagttctg aagtcacttg gttccgtatt
2761 gttttcgtcc tcttctcaa gcaacgattc ttgtctaagc ttattcaacg gtaccaaaaga
55 2821 cccgagtcct tttatgagag aaaacatttc atcatttttc aactcaatta tcttaatatc
2881 atttgtagt attttgaata caggatggta aaacgaatca cctgaatcta gaagctgtac
2941 cttgtcccat aaaagtttta atttactgag ctttctcggtc aagtaaaacta gtttatctag
3001 ttttgaaccg aatattgtgg gcagatttgc agtaagttca gttagatcta ctaaaagttg

```

3061 ttgacagca gccgattcca caaaaatttg gtaaaaggag atgaaagaga cctcgcgct
 3121 aatggtttgc atcaccatcg gatgtctgtt gaaaaactca ctttttgc at ggaagttatt
 3181 aacaataaga ctaatgatta cttagaata atgtataa

5 Yeast MLH1 protein (accession number U07187)

MSLRKALDASVVKIAAGEIIISPVNALKEMMENSIDANATMI
 10 DILVKEGGIKVLQITDNGSGINKADLPILCERFTTSKLQKFEDLSQIQTYGFRGEALA
 SISHVARVTVTTKVKEDRCARVSYAEGKMLESPPKPVAGKDGTTLVEDLFFNIPSR
 RALRSHNDEYSKILDVVGRYAIHSDKIGFSCKKFGDSNYSLSVKPSYTVQDRIRTVFN
 15 KSVASNLITFHISKVEDLNLESVDGKVCNLFISKKSISLIFFINNRLVTCDLLRRAL
 NSVYSNYLPKGFRPFYILGIVIDPAAVDVNVHPTKREVRFLSQDEIIIEKIANQLHAEL
 20 SAIDTSRTFKASSISTNKPESLIPFNDTIESDRNRKSLRQAQVVENSYTTANSQLRKA
 KRQENKLVRIDASQAKITSFLSSSQQNFEGSSSTRQLEPKVTNVSHSQEAEKLTN
 ESEQPRDANTINDNDLKDQPKKKQKLGDKVPSIADDEKNALPISKDGYIRVPKERN
 25 VNLTSIKKLREKVDDSIHRELTDFANLNYGVVDEERRLAIIQHDLKLFLIDYGSVC
 YELFYQIGLTFANFGKINLQSTNVSDDIVLYNLLSEFDELNDASKEKIIISKIWDMS
 30 SMLNEYYSIELVNDGLDNDLKS VKLKSPLLLKGYIPSLVKLPFFIYRLGKEVDWEDE
 QECLDGILREIALLYIPDMVPKVDTLASLSEDEKAQFINRKEHISLLEHVLFP
 RRFLAPRHILKDVVEIANLPDLYKVFERC

35 Mouse PMS2 protein

MEQTEGVSTE CAKAIKPIDG KSVHQICSGQ VILSLSTAVK ELIENSVDAG ATTIDRLRKD 60
 YGVDLIEVSD NGCGVEEENF EGLALKHHTS KIQEFADLTQ VETFGFRGEA LSSLCALSDV 120
 TISTCHGSAS VGTRLVFDHN GKITQKTPYP RPKGTTVSQ HLFYTLPVRY KEFQRIKKE 180
 YSKMVQVLQA YCIISAGVRV SCTNQLGQK RHAVVCTSGT SGMKENIGSV FGQKQLQSLI 240
 40 PFVQLPPSDA VCEEYGLSTS GRHKTFTFR ASFHSARTAP GGVQQTGSFS SSIRGPVTQQ 300
 RSLSLSMRFY HMYNRHQYPF VVLNVSDSE CVDINVTDPK RQILLQEEKL LLAVLKTS LI 360
 GMFSDANKL NVNQOPLLDV EGNLVKLHTA ELEKPVPGKQ DNSPSLKSTA DEKRVASIS 420
 LREAFSLHPT KEIKSRGPET AELTRSPSE KRGVLSSYPS DVISYRGLRG SQDKLVSPD 480
 SPGDCMDREK IEKDSGLSST SAGSEEEFST PEVASSFSSD YNVSSLEDRP SQETINCGDL 540
 45 DCRPPGTGQS LKPEDHGYQC KALPLARLSP TNAKRFKTEE RPSNVNISQR LPPGPQSTSAA 600
 EVDVAIKMNK RIVLLEFSL SLAKRMKQLQ HLKAQNKHEL SYRKFRKIC PGENQAAEDE 660
 LRKEISKSMF AEMEILGQFN LGFIVTKLKE DLFLVDQHAA DEKYNFEMLO QHTVLQAQRL 720
 ITPQTLNLTA VNEAVLIENL EIFRKNQFDF VIDEDAPVTE RAKLISLPTS KNWTFGPQDI 780
 DELIFMLSDS PGVMCRPSRV RQMFASRACR KSMIGTALN ASEMKKLITH MGEMDHPWNC 840
 50 PHGRPTMRHV ANLDVISQN 859

Mouse PMS2 cDNA

gaattccggt gaaggtcctg aagaatttcc agattcctga gtatcattgg aggagacaga 60
 taacctgtcg tcaggtaacg atggtgtata tgcaacagaa atgggtgttc ctggagacgc 120
 55 gtcttttccc gagagcggca ccgcaactct cccgcggtga ctgtgactgg aggagtctg 180
 catccatgga gcaaaccgaa ggcgtgagta cagatgtgc taaggccatc aagcctattg 240

atgggaagtc agtccatcaa atttggtctg ggcaggtgat actcagttta agcaccgctg 300
 tgaaggagtt gatagaaaat agtgtagatg ctggtgctac tactattgat ctaaggctta 360
 aagactatgg ggtggacctc attgaagttt cagacaatgg atgtgggta gaagaagaaa 420
 actttgaagg tctagctctg aaacatcaca catctaagat tcaagagttt gccgacctca 480
 5 cgcaggttga aactttcggc tttcgggggg aagctctgag ctctctgtgt gcaactaagtg 540
 atgtcactat atctacctgc cacgggtctg caagcgttgg gactcgactg gtgtttgacc 600
 ataatgggaa aatcacccag aaaactccct acccccgacc taaaggaacc acagtcaagtg 660
 tgcagcactt attttataca ctaccctgct gttacaaaga gtttcagagg aacattaaaa 720
 aggagtattc caaaatggtg caggtcttac aggcgtactg tatcatctca gcaggcgtcc 780
 10 gtgtaagctg cactaatcag ctcgacaggg ggaagcggca cgctgtggtg tgcacaagcg 840
 gcacgtctgg catgaaggaa aatatcgggt ctgtgtttgg ccagaagcag ttgcaaagcc 900
 tcatttcctt tgttcagctg ccccttagtg acgctgtgtg tgaagagtac ggcctgagca 960
 cttcaggacg ccacaaaacc ttttctacgt ttcgggcttc atttcacagt gcacgcacgg 1020
 cgccgggagg agtgcaacag acaggcagtt tttcttcac aatcagaggc cctgtgaccc 1080
 15 agcaaaggtc tctaagcttg tcaatgaggt tttatcacat gtataaccgg catcagtacc 1140
 catttgctgt ccttaacgtt tccgttgact cagaatgtgt ggatattaat gtaactccag 1200
 ataaaaggca aattctacta caagaagaga agctattgct ggccgtttta aagacctcct 1260
 tgataggaat gtttgacagt gatgcaaaca agcttaatgt caaccagcag ccactgctag 1320
 atgttgaagg taacttagta aagctgcata ctgcagaact agaaaagcct gtgccaggaa 1380
 20 agcaagataa ctctccttca ctgaagagca cagcagacga gaaaagggtg gcatccatct 1440
 ccaggctgag agaggccttt tctcttcac ctactaaaga gatcaagtct aggggtccag 1500
 agactgctga actgacacgg agttttccaa gtgagaaaag gggcgtgtta tcctcttacc 1560
 cttcagacgt catctcttac agaggcctcc gtggctcgca ggacaaattg gtgagtccca 1620
 cggacagccc tgggtgactgt atggacagag agaaaataga aaaagactca gggctcagca 1680
 25 gcacctcagc tggctctgag gaagagttca gcacccaga agtggccagt agcttttagca 1740
 gtgactataa cgtgagctcc ctagaagaca gaccttctca ggaaaccata aactgtggtg 1800
 acctggactg ccgtctcca ggtacaggac agtccttgaa gccagaagac catggatata 1860
 aatgcaaagc tctacctcta gctcgtctgt caccacaaa tgccaagcgc ttcaagacag 1920
 aggaaagacc ctcaaagtgc aacatttctc aaagattgcc tggtcctcag agcacctcag 1980
 30 cagctgaggt cgatgtagcc ataaaaatga ataagagaat cgtgctctc gagttctctc 2040
 tgagttctct agctaagcga atgaagcagt tacagcacct aaaggcgcag aacaaacatg 2100
 aactgagtta cagaaaattt agggccaaga tttgccctgg agaaaaccaa gcagcagaag 2160
 atgaactcag aaaagagatt agtaaatega tgtttgcaga gatggagatc ttgggtcagt 2220
 ttaacctggg atttatagta accaaactga aagaggacct cttcctggtg gaccagcatg 2280
 35 ctgcggatga gaagtacaac tttgagatgc tgcagcagca cacggtgctc caggcgcaga 2340
 ggctcatcac accccagact ctgaacttaa ctgctgtcaa tgaagctgta ctgatagaaa 2400
 atctggaaat attcagaaaag aatggctttg actttgtcat tgatgaggat gctccagtca 2460
 ctgaaagggc taaattgatt tccttaccaa ctagtaaaaa ctggaccttt ggaccccaag 2520
 atatagatga actgatcttt atgttaagtg acagccctgg ggtcatgtgc cggccctcac 2580
 40 gagtcagaca gatgtttgct tccagagcct gtcggaagtc agtgatgatt ggaacggcgc 2640

tcaatgcgag cgagatgaag aagctcatca cccacatggg tgagatggac cacccttggg 2700
 actgccccca cggcaggcca accatgaggc acgttgccaa tctggatgtc atctctcaga 2760
 actgacacac cccttgtagc atagagtta ttacagattg ttcggtttgc aaagagaagg 2820
 ttttaagtaa tctgattatc gttgtacaaa aattagcatg ctgctttaat gtactggatc 2880
 5 catttaaaag cagtgttaag gcaggcatga tggagtgttc ctctagctca gctacttggg 2940
 tgatccggtg ggagctcatg tgagcccagg actttgagac cactccgagc cacattcatg 3000
 agactcaatt caaggacaaa aaaaaaaga tatttttgaa gccttttaaa aaaaaa 3056

human PMS2 protein

10 MKQLPAATVR LLSSSQIITS VVSUVKELIE NSLDAGATSV DVKLENYGFD KIEVRDNGEG 60
 IKAVDAPVMA MKYYTSKINS HEDLENLTTY GFRGEALGSI CCIAEVLITT RTAADNFSTQ 120
 YVLDGSGHIL SQKPSHLGQG TTVTALRLFK NLPVRKQFYS TAKKCKDEIK KIQDLLMSFG 180
 ILKPDLRIVF VHNKAVIWQK SRVSDHKMAL MSVLGTAVMN NMESFQYHSE ESQIYLSGFL 240
 PKCDADHSFT SLSTPERSFI FINSRPVHOK DILKLIRHHY NLKCLKESTR LYPVFFLKID 300
 15 VPTADVDVNL TPKSQVLLQ NKESVLIALL NLMTTCYGPL PSTNSYENNK TDVSAADIVL 360
 SKTAETDVLV NKVESSGKNY SNVDTSVIPF QNDMHNDSEG KNTDDCLNHQ ISIGDFGYGH 420
 CSSEISNIDK NTKNAFQDIS MSNVSWENSQ TEYSKTCFIS SVKHTQSENG NKDHIDESGE 480
 NEEEAGLENS SEISADEWSR GNILKNSVGE NIEPVKILVP EKSLPCKVSN NNYPPEQMN 540
 LNEDESCNKK NVIDNKGKV TAYDLLSNRV IKKPMASAL FVQDHRPQFL IENPKTSLED 600
 20 ATLQIEELWK TLSEEEKLKY EEKATKDLER YNSQMKRAIE QESQMSLKDQ RKKIKPTSAR 660
 NLAQKHKLKT SLSNQPKLDE LLQSQIEKRR SQNIKMVQIP FSMKNLKNF KKQNKVDLEE 720
 KDEPCLIHNL RFPDAWLMTS KTEVMLLNPY RVEEALLFKR LLENHKLPAE PLEKPIMLTE 780
 SLFNGSHYLD VLYKMTADDQ RYSGSTYLSL PRLTANGFKI KLIPGVSITE NYLEIEGMAN 840
 CLPFYGVADL KEILNAILNR NAKVEYECRP RKVISYLEGE AVRLSRQLPM YLSKEDIQDI 900
 25 IYRMKHQFGN EIKECVHGRP FFHHLTYLPE TT 932

30

Human PMS2 cDNA

cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60
 aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcagggtgga 120
 35 ctgagtctaa gcaactgcgt aaaggagtta gtagaaaaca gtctggatgc tgggtgccact 180
 aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240
 tgtggggtag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
 caagagtgtg ccgacctaac tcaggttgaa acttttggct ttcgggggga agctctgagc 360
 tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaagggttga 420
 40 actcgactga tgtttgatca caatgggaaa attatccaga aaaccacctc ccccgcccc 480

```

agagggacca cagtcagcgt gcagcagtta tttccacac tacctgtgcg ccataaggaa 540
tttcaaagga atattaagaa ggagtatgcc aaaatggtcc aggtcttaca tgcatactgt 600
atcatttcag caggcatccg tgtaagttgc accaatcagc ttggacaagg aaaacgacag 660
cctgtggtat gcacaggtgg aagccccagc ataaaggaaa atatcggctc tgtgtttggg 720
5  cagaagcagt tgcaaagcct cattcctttt gttcagctgc cccctagtga ctccgtgtgt 780
gaagagtacg gtttgagctg ttcggatgct ctgcataatc ttttttacat ctccaggtttc 840
atttcacaat gcacgcatgg agttggaagg agttcaacag acagacagtt tttctttatc 900
aaccggcggc cttgtgaccc agcaaaggtc tgcagactcg tgaatgaggt ctaccacatg 960
tataatcgac accagtatcc atttgttgtt cttaacattt ctgttgattc agaatgcgtt 1020
10 gatatcaatg ttactccaga taaaaggcaa attttgctac aagaggaaaa gcttttgttg 1080
gcagttttaa agacctcttt gataggaatg tttgatagtg atgtcaacaa gctaaatgtc 1140
agtcagcagc cactgctgga tgttgaagggt aacttaataa aaatgcatgc agcggatttg 1200
gaaaagccca tggtagaaaa gcaggatcaa tccccctcat taaggactgg agaagaaaaa 1260
aaagacgtgt ccatttcag actgcgagag gccttttctc ttcgtcacac aacagagaac 1320
15 aagcctcaca gccc aaagac tccagaacca agaaggagcc ctctaggaca gaaaaggggt 1380
atgctgtctt ctagcacttc aggtgccatc tctgacaaag gcgtcctgag acctcagaaa 1440
gaggcagtga gttccagtca cggaccaggt gaccctacgg acagagcgga ggtggagaag 1500
gactcggggc acggcagcac ttcctggtat tctgaggggt tcagcatccc agacacgggc 1560
agtactgca gcagcgagta tgcggccagc tccccagggg acaggggctc gcaggaacat 1620
20 gtggactctc aggagaaagc gcctgaaact gacgactctt tttcagatgt ggactgccat 1680
tcaaaccagg aagataccgg atgtaaattt cgagttttgc ctcagccaac taatctcgca 1740
accccaaca caaagcgttt taaaaagaa gaaattcttt ccagttctga catttgtcaa 1800
aagttagtaa atactcagga catgtcagcc tctcaggttg atgtagctgt gaaaattaat 1860
aagaaagttg tgcccctgga cttttctatg agttctttag ctaaacgaat aaagcagtta 1920
25 catcatgaag cacagcaaag tgaaggggaa cagaattaca ggaagtttag ggcaaagatt 1980
tgtcctggag aaaatcaagc agccgaagat gaactaagaa aagagataag taaaacgatg 2040
tttgagaaaa tggaaatcat tggtcagttt aacctgggat ttataataac caaactgaat 2100
gaggatatct tcatagtgga ccagcatgcc acggacgaga agtataactt cgagatgctg 2160
cagcagcaca ccgtgtctca ggggcagagg ctcatagcac ctcagactct caacttaact 2220
30 gctgttaatg aagctgttct gatagaaaat ctggaaatat ttagaaagaa tggctttgat 2280
tttgttatcg atgaaaatgc tccagtcact gaaagggcta aactgatttc cttgccaaact 2340
agtaaaaact ggaccttcgg accccaggac gtcgatgaac tgatcttcat gctgagcgac 2400
agccctgggg tcatgtgccg gccttcccga gtcaagcaga tgtttgccctc cagagcctgc 2460
cggaagtcgg tgatgattgg gactgctctt aacacaagcg agatgaagaa actgatcacc 2520
35 cacatggggg agatggacca cccctggaac tgtcccatg gaaggccaac catgagacac 2580
atcgccaacc tgggtgtcat ttctcagaac tgaccgtagt cactgtatgg aataattggg 2640
tttatcgag atttttatgt tttgaaagac agagtcttca ctaacctttt ttgttttaaa 2700
atgaaacctg ctacttaaaa aaaatacaca tcacacccat ttaaaagtga tcttgagaac 2760
cttttcaaac c 2771
40

```


human PMS1 protein	
	MKQLPAATVR LLSSSQIITS VVSVMKELIE NSLDAGATSV DVKLENYGFD KIEVRDNGEG 60
	IKAVDAPVMA MKYYTSKINS HEDLENLTTY GFRGEALGSI CCIAEVLITT RTAADNFSTQ 120
5	YVLDGSGHIL SQKPSHLGQG TTVTALRLFK NLPVRKQFYS TAKKCKDEIK KIQDLLMSFG 180
	ILKPDRLRIV VHNKAVIWQK SRVSDHKMAL MSVLGTAVMN NMESFQYHSE ESQIYLSGFL 240
	PKCDADHSFT SLSTPERSFI FINSRPVHOK DILKLIRHHY NLKCLKESTR LYPVFFLKID 300
	VPTADVVDNL TPKDSQVLLQ NKESVLIALE NLMTTCYGPL PSTNSYENNK TDVSAADIVL 360
	SKTAETDVLV NKVESSGKNY SNVDTSVIPF QNDMHNDESG KNTDDCLNHQ ISIGDFGYGH 420
10	CSSEISNIDK NTKNAFQDIS MSNVSWENSQ TEYSKTCFIS SVKHTQSENG NKDHIDESGE 480
	NEEEAGLENS SEISADEWSR GNILKNSVGE NIEPVKILVP EKSLPCKVSN NNYPIPEQMN 540
	LNEDSCNKKK NVIDNKSGKV TAYDLLSNRV IKKPMASAL FVQDHRPQFL IENPKTSLED 600
	ATLQIEELWK TLSEEEKLKY EEKATKDLER YNSQMKAIE QESQMSLKDG RKKIKPTSAA 660
	NLAQKHKLKT SLSNQPKLDE LLQSQIEKRR SQNIKMVQIP FSMKNLKINF KKQNKVDLEE 720
15	KDEPCLIHNL RFPDAWLMTS KTEVMLLNPY RVEEALLFKR LLENHKLPAE PLEKPIMLTE 780
	SLFNGSHYLD VLYKMTADDQ RYSGSTYLSL PRLTANGFKI KLIPGVSITE NYLEIEGMA 840
	CLPFYGVADL KEILNAILNR NAKEVYECRP RKVISYLEGE AVRLSRQLPM YLSKEDIQDI 900
	IYRMKHQFGN EIKECVHGRP FFHHLTYLPE TT 932
20 Human PMS1 cDNA	
	ggcagcagtg gctgcttgcg gctagtggtt ggtaattgcc tgcctcgcg tagcagcaag 60
	ctgctctgtt aaaagcgaaa atgaaacaat tgcctcgcg aacagttcga ctcccttcaa 120
	gttctcagat catcacttcg gtggtcagtg ttgtaaaaga gcttattgaa aactccttgg 180
	atgctggtgc cacaagcgta gatgttaaac tggagaacta tggatttgat aaaattgagg 240
25	tgcgagataa cggggagggt atcaaggctg ttgatgcacc tgtaatggca atgaagtact 300
	acacctcaaa aataaatagt catgaagatc ttgaaaattt gacaacttac ggttttcgtg 360
	gagaagcctt ggggtcaatt tgttgtatag ctgaggtttt aattacaaca agaacggctg 420
	ctgataattt tagcaccag tatgttttag atggcagtg ccacatactt tctcagaaac 480
	cttcacatct tgggtcaagg acaactgtaa ctgctttaag attatttaag aatctacctg 540
30	taagaaagca gttttactca actgcaaaaa aatgtaaaaga tgaaataaaa aagatccaag 600
	atctcctcat gagctttggt atccttaaac ctgacttaag gattgtcttt gtacataaca 660
	aggcagttat ttggcagaaa agcagagtat cagatcacaa gatggctctc atgtcagttc 720
	tggggactgc tgttatgaac aatatggaat cctttcagta ccactctgaa gaatctcaga 780
	tttatctcag tggatttctt ccaaagtgtg atgcagacca ctctttcact agtctttcaa 840
35	caccagaaag aagtttcatc ttcataaaca gtcgaccagt acatcaaaaa gatattcttaa 900
	agttaatccg acatcattac aatctgaaat gcctaaagga atctactcgt ttgtatcctg 960
	ttttcttctt gaaaatcgat gttcctacag ctgatgttga tgtaaattta acaccagata 1020
	aaagccaagt attattacaa aataaggaat ctgttttaat tgctcttgaa aatctgatga 1080
	cgacttggtt tggaccatta cctagtacaa attcttatga aaataataaa acagatgttt 1140
40	ccgcagctga catcgttctt agtaaaacag cagaaacaga tgtgcttttt aataaagtgg 1200

aatcatctgg aaagaattat tcaaatgttg atacttcagt cattccattc caaaatgata 1260
 tgcataatga tgaatctgga aaaaacactg atgattgttt aaatcaccag ataagtattg 1320
 gtgactttgg ttatggtcat tgtagtagtg aaatttctaa cattgataaa aacactaaga 1380
 atgcatttca ggacatttca atgagtaatg tatcatggga gaactctcag acggaatata 1440
 5 gtaaaaacttg tttataagt tccgttaagc acaccagtc agaaaatggc aataaagacc 1500
 atatagatga gagtggggaa aatgaggaag aagcaggtct tgaaaactct tcggaaattt 1560
 ctgcagatga gtggagcagg ggaaatatac ttaaaaattc agtgggagag aatattgaac 1620
 ctgtgaaaat tttagtgcct gaaaaaagtt taccatgtaa agtaagtaat aataattatc 1680
 caatccctga acaaatgaat cttaatgaag attcatgtaa caaaaaatca aatgtaatag 1740
 10 ataataaatc tggaaaagtt acagcttatg atttacttag caatcgagta atcaagaaac 1800
 ccatgtcagc aagtgtctct tttgttcaag atcatcgtcc tcagtttctc atagaaaatc 1860
 ctaagactag tttagaggat gcaacactac aaattgaaga actgtggaag acattgagtg 1920
 aagaggaaaa actgaaatat gaagagaagg ctactaaaga cttggaacga tacaatagtc 1980
 aaatgaagag agccattgaa caggagtcac aaatgtcact aaaagatggc agaaaaaaga 2040
 15 taaaaccac cagcgcatgg aatttggccc agaagcaca gttaaaaacc tcattatcta 2100
 atcaaccaa acttgatgaa ctcttcagt cccaaattga aaaaagaagg agtcaaaata 2160
 ttaaaatgg acagatcccc ttttctatga aaaacttaa aataaatttt aagaaacaaa 2220
 acaaagttga cttagaagag aaggatgaac cttgcttgat ccacaatctc aggtttcctg 2280
 atgcatggct aatgacatcc aaaacagagg taatgttatt aaatccatat agagtagaag 2340
 20 aagccctgct atttaaaaga cttcttgaga atcataaact tcctgcagag ccactggaaa 2400
 agccaattat gttaacagag agtcttttta atggatctca ttatttagac gttttatata 2460
 aaatgacagc agatgacca agatacagtg gatcaactta cctgtctgat cctcgtctta 2520
 cagcgaatgg tttcaagata aaattgatac caggagtctc aattactgaa aattacttgg 2580
 aaatagaagg aatggcta atgtctccat tctatggagt agcagattta aaagaaattc 2640
 25 ttaatgctat attaaacaga aatgcaaagg aagtttatga atgtagacct cgcaaagtga 2700
 taagtatttt agaggagaa gcagtgcgtc tatccagaca attacccatg tacttatcaa 2760
 aagaggacat ccaagacatt atctacagaa tgaagcacca gtttggaat gaaattaaag 2820
 agtgtgttca tggtcgccc ttttttcac atttaaccta tcttcagaa actacatgat 2880
 taaatatgtt taagaagatt agttaccatt gaaattggtt ctgtcataaa acagcatgag 2940
 30 tctggtttta aattatcttt gtattatgtg tcacatggtt attttttaa tgaggattca 3000
 ctgacttggt tttatattga aaaaagttcc acgtattgta gaaaacgtaa ataaactaat 3060
 aac 3063

35 human MSH2 protein
 MAVQPKETLQ LESAAEVGFV RFFQGMPEKP TTVRLFDRG DFYTAHGEDA LLAAREVFKT 60
 QGVIKYMGPA GAKNLQSVVL SKMNFESFVK DLLLVQRQYRV EVYKNRAGNK ASKENDWYLA 120
 YKASPGNLSQ FEDILFGNND MSASIGVVGK KMSAVDGQRQ VGVGYVDSIQ RKLGLCEFPD 180
 NDQFSNLEAL LIQIGPKECV LPGGETAGDM GKLRQIIQRG GILITERKKA DFSTKDIYQD 240
 40 LNRLKKGKKG EQMNSAVLPE MENQVAVSSL SAVIKFLELL SDDSNFGQFE LTTDFDSQYM 300

KLDIAAVRAL NLFQGSVEDT TGSQSLAALL NKCKTPQGQR LVNQWIKQPL MDKNRIEERL 360
 NLVEAFVEDA ELRQTLQEDL LRRFPDLNRL AKKFQRQAAN LQDCYRLYQG INQLPNVIQA 420
 LEKHEGKHQK LLLAVFVTPL TDLRSDFSKF QEMIETTLDM DQVENHEFLV KPSFDPNLSE 480
 LREIMNDLEK KMQSTLISAA RDLGLDPGKQ IKLDSSAQFG YYFRVTCKEE KVLRRNNKNFS 540
 5 TVDIQKNGVK FTNSKLTSLN EEYTKNKTEY EEAQDAIVKE IVNISSGYVE PMQTLNDVLA 600
 QLDVVVSFAH VSNAPVPYV RPAILEKGQG RIILKASRHA CVEVQDEIAF IPNDVYFEKD 660
 KQMFHIITGP NMGGKSTYIR QTGVIVLMAQ IGCVPCESA EVSIVDCILA RVGAGDSQLK 720
 GVSTFMAEML ETASILRSAT KDSLIIIDEL GRGTSTYDGF GLAWAISEYI ATKIGAFCMF 780
 ATHFHELTAL ANQIPTVNNL HVTALTTEET LTMLYQVKKG VCDQSFGIHV AELANFPKHV 840
 10 ICAKQKALE LEEFYIGES QGYDIMEPAA KKCYLEREQG EKIIQEFLSK VKQMPFTEMS 900
 EENITIKLKQ LKAETIAKNN SFVNEIISRI KVTT 934

Human MSH2 cDNA

ggcgggaaac agcttagtgg gtgtggggtc gcgcattttc ttcaaccagg aggtgaggag 60
 15 gtttcgacat ggcggtgcag ccgaaggaga cgctgcagtt ggagagcgcg gccgaggtcg 120
 gcttcgtgcg cttctttcag ggcagccgg agaagccgac caccacagtg cgccttttcg 180
 accggggcga cttctatacg gcgcacggcg aggacgcgct gctggccgcc cgggagggtg 240
 tcaagaccca gggggtgatc aagtacatgg ggccggcagg agcaaagaat ctgcagagtg 300
 ttgtgcttag taaaatgaat tttgaatcct ttgtaaaaga tcttcttctg gttcgtcagt 360
 20 atagagttga agtttataag aatagagctg gaaataaggc atccaaggag aatgattggt 420
 atttggcata taaggcttct cctggcaatc tctctcagtt tgaagacatt ctctttggta 480
 acaatgatat gtcagcttcc attggtggtg tgggtggtta aatgtccgca gttgatggcc 540
 agagacaggt tggagttggg tatgtggatt ccatacagag gaaactagga ctgtgtgaat 600
 tccctgataa tgatcagttc tccaatcttg aggtctctct catccagatt ggaccaaagg 660
 25 aatgtgtttt acccgaggga gagactgctg gagacatggg gaaactgaga cagataattc 720
 aaagaggagg aattctgatc acagaaagaa aaaaagctga cttttccaca aaagacattt 780
 atcaggacct caaccggttg ttgaaaggca aaaagggaga gcagatgaat agtgcgtgat 840
 tgccagaaat ggagaatcag gttgcagttt catcactgtc tgcggtaatc aagtttttag 900
 aactcttatc agatgattcc aactttggac agtttgaact gactactttt gacttcagcc 960
 30 agtatatgaa attggatatt gcagcagtc gagcccttaa cctttttcag ggttctgttg 1020
 aagataccac tggctctcag tctctggtg ccttgctgaa taagtgtaaa acccctcaag 1080
 gacaaagact tgtaaccag tggattaagc agcctctcat ggataagaac agaatagagg 1140
 agagattgaa tttagtggaa gctttttag aagatgcaga attgaggcag actttacaag 1200
 aagatttact tcgtcgattc ccagatctta accgacttgc caagaagttt caaagacaag 1260
 35 cagcaaactt acaagattgt taccgactct atcaggggtat aaatcaacta cctaattgta 1320
 tacaggctct ggaaaaacat gaaggaaaac accagaaatt attgttggca gtttttgtga 1380
 ctctcttac tgatcttcgt tctgacttct ccaagtttca ggaaatgata gaaacaactt 1440
 tagatatgga tcagggtgaa aaccatgaat tccttgtaaa accttcattt gatcctaate 1500
 tcagtgaatt aagagaaata atgaatgact tggaaaagaa gatgcagtc acattaataa 1560
 40 gtgcagccag agatcttggc ttggaccctg gcaaacagat taaactggat tccagtgcac 1620

```

    agtttggata ttactttcgt gtaacctgta aggaagaaaa agtccttcgt aacaataaaa 1680
    acttttagtac tgtagatata cagaagaatg gtgttaaatt taccaacagc aaattgactt 1740
    ctttaaataga agagtatacc aaaaataaaa cagaatatga agaagcccag gatgccattg 1800
    ttaaagaaat tgtcaatatt tcttcaggct atgtagaacc aatgcagaca ctcaatgatg 1860
5   tgttagctca gctagatgct gttgtcagct ttgctcacgt gtcaaagga gacacctgttc 1920
    catatgtacg accagccatt ttggagaaa gacaaggaag aattatatta aaagcatcca 1980
    ggcatgcttg tgttgaagtt caagatgaaa ttgcatttat tcctaataac gtatactttg 2040
    aaaaagataa acagatgttc cacatcatta ctggcccaaa tatgggaggt aaatcaacat 2100
    atattcgaca aactggggtg atagtactca tggcccaaat tgggtgtttt gtgccatgtg 2160
10  agtcagcaga agtgtccatt gtggactgca tcttagcccg agtaggggct ggtgacagtc 2220
    aattgaaagg agtctccacg ttcattggctg aaatgttggg aactgcttct atcctcaggt 2280
    ctgcaaccaa agattcatta ataatcatag atgaattggg aagaggaact tctacctacg 2340
    atggatttgg gttagcatgg gctatatcag aatacattgc aacaaagatt ggtgcttttt 2400
    gcatgtttgc aaccattttt catgaactta ctgccttggc caatcagata ccaactgtta 2460
15  ataatctaca tgtcacagca ctcaccactg aagagacett aactatgctt tatcagggtga 2520
    agaaagggtgt ctgtgatcaa agttttggga ttcattgttc agagcttgct aatttcccta 2580
    agcatgtaat agagtgtgct aaacagaaa gacctggaact tgaggagttt cagtatattg 2640
    gagaatcgca aggatatgat atcatggaac cagcagcaaa gaagtgcctat ctggaaagag 2700
    agcaagggtga aaaaattatt caggagttcc tgtccaaggt gaaacaaatg ccctttactg 2760
20  aaatgtcaga agaaaacatc acaataaagt taaaacagct aaaagctgaa gtaatagcaa 2820
    agaataatag ctttgtaaat gaaatcattt cacgaataaa agttactacg tgaaaaatcc 2880
    cagtaatgga atgaaggtaa tattgataag ctattgtctg taatagtttt atattgtttt 2940
    atattaaccc tttttccata gtgttaactg tcagtgccca tgggctatca acttaataag 3000
    atatttagta atattttact ttgaggacat tttcaaagat ttttattttg aaaaatgaga 3060
25  gctgtaactg aggactgttt gcaattgaca taggcaataa taagtgatgt gctgaatttt 3120
    ataaataaaa tcatgtagtt tgtgg                                     3145

```

human MLH1 protein

```

    MSFVAGVIRR LDETIVNRIA AGEVIQRPAN AIKEMIENCL DAKSTSIQVI VKEGGLKLIQ 60
30  IQDNGTGIRK EDLDIVCERF TTSKLQSFED LASISTYGFR GEALASISHV AHVTITTKTA 120
    DGKCAIRASY SDGKLKAPPK PCAGNQGTQI TVEDLFYNIA TRRKALKNPS EEEYKILEVV 180
    GRYSVHNAGI SFSVKKQGET VADVRTLPA STVDNIRSIF GNAVSRELIE IGCEDKTLAF 240
    KMNGYISNAN YSVKKCIFLL FINHRLVEST SLRKAIETVY AAYLPKNTHP FLYLSLEISP 300
    QNV DVNVHPT KHEVHFLHEE SILERVQQHI ESKLLGSNSS RMYFTQTLLP GLAGPSGEMV 360
35  KSTTSLTSSS TSGSSDKVYA HQMVRTDSRE QKLDAFLQPL SKPLSSQPQA IVTEDKTDIS 420
    SGRARQQDEE MLELPAPAEV AAKNQSLEGD TTKGTSEMSE KRGPTSSNPR KHRHRESDVE 480
    MVEDDSRKEM TAACTPRRRI INLTSVLSLQ EEINEQGHEV LREMLHNHSF VGCVPNPQWAL 540
    AQHQTKLYLL NTKLSEELF YQILIYDFAN FGVRLRLEPA PLFDLAMLAL DSPESGWTEE 600
    DGPKEGLAEY IVEFLKKKAE MLADYFSLEI DEEGNLIGLP LLIDNYVPPL EGLPIFILRL 660
40  ATEVNWDEEK ECFESLSKEC AMFYISIRKQY ISEESTLSGQ QSEVPGSIPN SWKWTVEHIV 720

```

YKALRSHILP PKHFTEDGNI LQLANLPDLY KVFERC

756

Human MLH1 cDNA

cttggctctt ctggcgccaa aatgtcggtc gtggcagggg ttattcggcg gctggacgag 60
 5 acagtgggtga accgcacgcg ggcgggggaa gttatccagc ggccagctaa tgctatcaaa 120
 gagatgattg agaactgttt agatgcaaaa tccacaagta ttcaagtgat tgttaaagag 180
 ggaggcctga agttgattca gatccaagac aatggcaccg ggatcaggaa agaagatctg 240
 gatattgtat gtgaaagggt cactactagt aaactgcagt cctttgagga tttagccagt 300
 atttctacct atggctttcg aggtgaggct ttggccagca taagccatgt ggctcatgtt 360
 10 actattacaa cgaaaacagc tgatggaaag tgtgcataca gagcaagtta ctcatgatgga 420
 aaactgaaag cccctcctaa accatgtgct ggcaatcaag ggacccagat cacggtggag 480
 gacctttttt acaacatagc cacgaggaga aaagctttaa aaaatccaag tgaagaatat 540
 gggaaaattt tggaaagtgt tggcaggtat tcagtacaca atgcaggcat tagtttctca 600
 gttaaaaaac aaggagagac agtagctgat gttaggacac taccatgc ctcaaccgtg 660
 15 gacaatatc gctccatctt tggaaatgct gttagtcgag aactgataga aattggatgt 720
 gaggataaaa ccctagcctt caaaatgaat ggttacatat ccaatgcaaa ctactcagt 780
 aagaagtga tcttcttact ctcatcaac catcgctctg tagaatcaac ttccttgaga 840
 aaagccatag aaacagtgt tgcagcctat ttgccccaaa acacacaccc attcctgtac 900
 ctcatgttag aaatcagtc ccagaatgtg gatgttaatg tgcacccac aaagcatgaa 960
 20 gttcacttcc tgcacgagga gagcatcctg gagcgggtgc agcagcacat cgagagcaag 1020
 ctctgggct ccaattctc caggatgtac ttcacccaga ctttgcctacc aggacttgc 1080
 ggccccctc gggagatggt taaatccaca acaagtctga cctcgtcttc tacttctgga 1140
 agtagtgata aggtctatgc ccaccagatg gtccgtacag attcccggga acagaagctt 1200
 gatgcatttc tgcagcctc gagcaaaccc ctgtccagtc agccccaggc cattgtcaca 1260
 25 gaggataaga cagatatttc tagtggcagg gctaggcagc aagatgagga gatgcttgaa 1320
 ctcccagccc ctgctgaagt ggctgccaaa aatcagagct tggaggggga tacaacaaag 1380
 gggacttcag aaatgtcaga gaagagagga cctacttcca gcaacccag aaagagacat 1440
 cggaagatt ctgatgtgga aatggtggaa gatgattccc gaaaggaaat gactgcagct 1500
 tgtaccccc ggagaaggat cattaacctc actagtgtt tgagtctcca ggaagaaatt 1560
 30 aatgagcagg gacatgaggt tctccgggag atgttgcata accactcctt cgtgggctgt 1620
 gtgaatctc agtgggcctt ggcacagcat caaaccaagt tataccttct caacaccacc 1680
 aagcttagtg aagaactgtt ctaccagata ctcatctatg attttgccaa ttttggtgtt 1740
 ctcaggttat cggagccagc accgctcttt gaccttgcca tgcctgcctt agatagtcca 1800
 gagagtggct ggacagagga agatggctcc aaagaaggac ttgctgaata cattgttgag 1860
 35 tttctgaaga agaaggctga gatgcttgca gactatttct ctttggaat tgatgaggaa 1920
 gggaaacctga ttggattacc ctttctgatt gacaactatg tgccccctt ggagggactg 1980
 cctatcttca ttcttcgact agccactgag gtgaattggg acgaagaaaa ggaatgtttt 2040
 gaaagcctca gtaagaatg cgctatgttc tattccatcc ggaagcagta catatctgag 2100
 gagtgcaccc tctcaggcca gcagagtga gtgcctggct ccattccaaa ctctggaag 2160
 40 tggactgtgg aacacattgt ctataaagcc ttgcgctcac acattctgcc tcctaaacat 2220

ttcacagaag atggaaatat cctgcagctt gctaacctgc ctgatctata caaagtcttt 2280
 gagagggtgtt aaatatggtt atttatgcac tgtgggatgt gttcttcttt ctctgtattc 2340
 cgatacaaag tgttgtatca aagtgtgata tacaaagtgt accaacataa gtgttggttag 2400
 cacttaagac ttatacttgc cttctgatag tattccttta tacacagtgg attgattata 2460
 5 aataaataga tgtgtcttaa cata 2484

hPMS2-134 protein

MKQLPAATVR LLSSSQIITS VVSUVKELIE NSLDAGATSV DVKLENYGFD KIEVRDNGEG 60
 IKAVDAPVMA MKYYTSKINS HEDLENLTYY GFRGEALGSI CCIAEVLITT RTAADNFSTQ 120
 10 YVLDGSGHIL SQK 133

hPMS2-134 cDNA

cgaggcggat cgggtgttgc atccatggag cgagctgaga gctcgagtac agaacctgct 60
 aaggccatca aacctattga tcggaagtca gtccatcaga tttgctctgg gcagggtggtta 120
 15 ctgagtctaa gcactgcggt aaaggagtta gtagaaaaca gtctggatgc tggtgccact 180
 aatattgatc taaagcttaa ggactatgga gtggatctta ttgaagtttc agacaatgga 240
 tgtggggttag aagaagaaaa cttcgaaggc ttaactctga aacatcacac atctaagatt 300
 caagagtttg ccgacctaac tcagggttgaa acttttggct ttcgggggga agctctgagc 360
 tcactttgtg cactgagcga tgtcaccatt tctacctgcc acgcatcggc gaaggttgga 420
 20 acttga 426

hMSH6 (human cDNA) ACCESSION U28946

MSRQSTLYSFFPKSPALSDANKASARASREGGAAAAAGASPPSP
 25 GGDAAWSEAGPGPRPLARSASPPKAKNLNGGLRRSVAPAAPTSCDFSPGDLVWAKMEG
 YPWWPCLVYNHPFDGTFIREKGKSVRVHVQFFDDSPTRGWVSKRLLKPYTGSKSKEAQ
 30 KGGHFYSAPKPEILRAMQRADEALNKDKIKRLELAVCDEPSEPEEEEEEMEVGTTYVTDK
 SEEDNEIESEEEVQPKTQGSRRSSRQIKKRRVISDSSESDIGGSDVEFKPDTKEEGSSD
 35 EISSGVGDSESEGLNSPVKVARKRKMVTGNGSLKRKSSRKETPSATKQATSISSETK
 NTLRAFSAPQNSSEQAHVSGGGDDSSRPTVWYHETLEWLKEEKRRDEHRRRPDHPDFD
 ASTLYVPEDFLNSCTPGMRKWWQIKSQNFDLVICYKVGKFYELYHMDALIGVSELGLV
 40 FMKGNWAHSGFPEIAFGRYSDSLVQGYKVARVEQTETPEMMEARCRKMAHISKYDRV
 VRREICRIITKGTQTYSVLEGDPSENYSKYLLSLKEKEEDSSGHTRAYGVCFVDTSLG
 45 KFFIGQFSDDRHCSRFRFTLVAHYPPVQVLFKGNLSKETKITLKSSLSCSLQEGLI PG
 SQFWDASKTLRTLLEEEYFREKLSDGIGVMLPQVLKGMTSESDSIGLTPGEKSELALS
 ALGGCVFYLLKKCLIDQELLSMANFEEYIPLDSDTVSTTRSGAIFTKAYQRMVLDVAVTL
 50 NNLEIFLNGTNGSTEGTLLERVDTCHTFPGKRLKQWLCAPLCNHYAINDRLDAIEDL
 MVVPDKISEVVELLKKLPDLERLLSKIHNVGSPKLSQNHPSRAIMYEETTYSKKKII
 55 DFLSALEGFKVMCKII GIMEEVADGFKSKILKQVISLQTKNPEGRFPDLTVELNRWD
 AFDHEKARKTGLITPKAGFSDYDQALADIRENEQSLLEYLEKQRNRIGCRTIVYWGI
 GRNRYQLEIPENFTTRNLPEEYELKSTKKGCKRYWTKTIEKKLANLINAERRDVS LK
 60 DCMRRLFYNFDKNYKDWQSAVECIAVL DVLLCLANYSRGGDGPMPVILLPEDTPPF

LELKGSRHPCITKTFFGDDFIPNDILIGCEEEQENGKAYCVLVTGPNMGGKSTLMRQ
 5 AGLLAVMAQMGCVPAEVCRLTPIDRVFTRLGASDRIMSGESTFFVELSETASILMHA
 TAHSLLVLVDELGRGTATFDGTAIANAVVKELAETIKRTRLFSTHYHSLVEDYSQNVAV
 10 RLGHMACMVENECEDPSQETITFLYKFIKGACPKSYGFNAARLANLPEEVIQKGRKA
 REF EKMNQSLRLFREVCLASERSTVD AEAVHKLLTLIKEL"

hPMSR2 (human cDNA) ACCESSION U38964

15 1 ggcgctccta cctgcaagtg gctagtgcca agtgctgggc cgccgctcct gccgtgcatg
 61 ttggggagcc agtacatgca ggtgggctcc acacggagag gggcgagac ccggtgacag
 121 ggctttacct ggtacatcgg catggcgcaa ccaaagcaag agagggtggc gcgtgccaga
 181 caccaacggt cggaaaccgc cagacaccaa cggtcggaaa ccgccaagac accaacgctc
 241 ggaaccgcgc agacaccaac gctcggaaa cgcagagac caaggctcgg aatccacgcc
 20 301 aggccacgac ggaggcgac tacctccctt ctgaccctgc tgctggcggt
 cggaaaaaac
 361 gcagtcgggt gtgctctgat tggccaggc tctttgacgt caccgactcg accttgaca
 421 gagccactag gcgaaaagga gagacgggaa gtattttttc cgccccgcc ggaaagggg
 481 gagcacaacg tcgaaagcag ccgttgggag ccagaggagc ggggcgcctg tgggagccgt
 25 541 ggaggggaact ttccagtc ccgagcgga tccggtgttg catccttga gcgagctgag
 601 aactcgagta cagaacctgc taaggccatc aaacctattg atcggaagtc agtccatcag
 661 atttgctctg gcccggtggt accgagtcta aggccgaatg cgggtgaagga gttagtagaa
 721 aacagtctgg atgctggtgc cactaatgtt gatctaaagc ttaaggacta tggagtggat
 781 ct cattgaag ttccaggcaa tggatgtggg gtagaagaag aaaacttcga aggccttact
 30 841 ctgaaacatc acacatgtaa gattcaagag ttgcccagacc taactcaggt ggaaactttt
 901 ggctttcggg gggaaactct gagctcactt tgtgactga gtgatgtcac catttctacc
 961 tgccgtgtat cagcgaaggt tgggactcga ctggtgtttg atcactatgg gaaaatcatc
 1021 cagaaaaccc cctacccccg cccagagggt atgacagtca gcgtgaagca gttattttct
 1081 acgctacctg tgaccataa agaatttcaa aggaatatta agaagaaacg tgctgtctc
 35 1141 cccttcgct tctgcgtga ttgtcagttt cctgaggcct cccagccat gcttctgta
 1201 cagcctgtag aactgactcc tagaagtacc ccacccacc cctgtcctt ggaggacaac
 1261 gtgactctg tattcagtc tgtcaagaat ggtccagggt cttctagat atctgcacaa
 1321 atgggttcctc tctccttcc tgatgtctgc cattagcatt ggaataaagt tctgtctgaa
 40 1381 aatccaaaaa aaaaaaaaaa aaaaaaaa

hPMSR2 (human protein) ACCESSION U38964

MAQPKQERVARARHQRSETARHQRSETAKTPTLGNRQPTLGNR
 45 QTPRLGIHARPRRRATTSLLTLLAFGKNAVRICALIGPSLTSRTRPLTEPLGEKERR
 EVFFPPRPERVEHNVESSRWEPRRRGACGSRGGNFSPRGGSGVASLERAENSSTEPA
 50 KAIKPIDRKSVHQICSGPVVPSLRPNAVKELVENS LDAGATNVDLKLDYGVVDLIEVS
 GNGCGVEEENFEGFTLKHHTCKIQEFADLTQVETFGFRGEALSSLCALSDVTISTCRV
 SAKVGTRLVFDHYGKIIQKTPYPRPGMTVSVKQLFSTLPVHHKEFQRNIKKKRACFP
 55 FAFCRDCQFPEASPAMLVPVQVELTPRSTPPHPCSLEDNVITVFSSVKNPGSSR

HPMSR3 (human cDNA) ACCESSION U38979

60 1 tttttagaaa ctgatgttta ttttccatca accatttttc catgctgctt aagagaatat
 61 gcaagaacag cttaagacca gtcagtgggt gctectacc attcagtggc ctgagcagtg
 121 ggagctgca gaccagtctt ccgtggcagg ctgagcgctc cagtcttcag tagggaattg

181 ctgaatagggc acagaggggca cctgtacacc ttcagaccag tctgcaacct caggetgagt
 241 agcagtgaaac tcaggagcgg gagcagtgcca ttcaccctga aattcctcct tgggtcactgc
 301 cttctcagca gcagcctgct cttctttttc aatctcttca ggatctctgt agaagtacag
 361 atcaggcatg acctcccatg ggtgttcacg ggaaatggtg ccacgcatgc gcagaacttc
 5 421 ccgagccagc atccaccaca ttaaaccacac tgagttagct cccttgttgt tgcattggat
 481 ggcaatgtcc acatagcgca gaggagaatc tgtgttacac agcgcaatgg taggtaggtt
 541 aacataagat gcctccgtga gaggcgaagg ggcggcgagg cccgggcctg gcccgatgt
 601 gtccttggcg gcctagacta ggccgtcgct gtatggtgag cccagggag gccgatctgg
 661 gccccagaa ggacacccgc ctggatttgc cccgtagccc ggcccgggccc cctcgaggagc
 10 721 agaacagcct tggtaggtg gacaggagg gacctcgca gcagacgcgc gcgccagcga
 781 cagcagcccc gccccggcct ctcgggagcc ggggggcaga ggctgcggag ccccaggagg
 841 gtctatcagc cacagtctct gcatgtttcc aagagcaaca ggaaatgaac acattgcagg
 901 ggccagtgtc attcaaagat gtggctgtgg atttcaccca ggaggagtgg cggcaactgg
 961 accctgatga gaagatagca tacggggatg tgatgttga gaactacagc catctagttt
 15 1021 ctgtggggta tgattatcac caagccaaac atcatcatgg agtggagggtg aagggaagtgg
 1081 agcagggaga ggagccgtgg ataatggaag gtgaatttcc atgtcaacat agtccagaac
 1141 ctgctaaggc catcaaacct attgatcga agtcagtcca tcagatttgc tctgggccag
 1201 tggtagtgag tctaagcact gcagtgaagg agttagtaga aaacagctctg gatgctgggtg
 1261 ccactaatat tgatctaaag cttaaggact atggagtga tctcattgaa gtttcagaca
 20 1321 atggatgtgg gtagaagaa gaaaactttg aaggcttaat ctctttcagc tctgaaacat
 1381 cacacatgta agattcaaga gtttgccgac ctaactgaag ttgaaacttt cggttttcag
 1441 ggggaagctc tgagctcact gtgtgcactg agcgatgtca ccatttctac ctgccacgcg
 1501 ttggtgaagg ttgggactcg actggtgttt gatcacgatg ggaaaatcat ccaggaaacc
 1561 ccctaccccc accccagagg gaccacagtc agcgtgaagc agttattttc tacgctacct
 25 1621 gtgcgccata aggaatttca aaggaatatt aagaagacgt gcctgcttcc ccttcgcctt
 1681 ctgccgtgat tgcagtttc ctgaggctc cccagccatg cttcctgtac agcctgcaga
 1741 actgtgagtc aattaaacct cttttcttca taaattaaaa aaaaa

30

hPMSR3 (human protein) ACCESSION U38979

MCPWRPRLGRRCMVSPREADLGPQKDRDLPRSPARAPREQNS

35 LGEVDRRGPREQTRAPATAAPPRPLGSRGAEAAEPQEGLSATVSACFQEQQEMNTLQG

PVSFKDVAVDFTQEEWRQLDPDEKIAYGDMLENYSHLVSVGYDYHQAKHHHGVEVKE

VEQGEEPWIMEGEFPCQHSPEPAKAIKPIDRKSVMHQCISGPVVLSTAVKELVENS

40

DAGATNIDLKLDYGVDLIEVSDNGCGVEEENFEGLISFSSETSHM"

hPMSL9 (human cDNA) ACCESSION NM_005395

45

1 atgtgtcctt ggcggcctag actaggccgt cgctgtatgg tgagccccag ggaggcggat
 61 ctggggcccc agaaggacac ccgcctggat ttgccccgta gcccgggccc ggcccctcgg
 121 gagcagaaca gccttgggtga ggtggacagg aggggacctc gcgagcagac gcgcgcgcca
 181 gcgacagcag ccccgccccg gcctctcggg agccgggggg cagaggctgc ggagccccag
 50 241 gaggggtctat cagccacagt ctctgcatgt ttccaagagc aacaggaaat gaacacattg
 301 cagggggccag tgcattcaa agatgtggct gtggatttca cccaggaggga gtggcgggcaa
 361 ctggaccctg atgagaagat agcatacggg gatgtgatgt tggagaacta cagccatcta
 421 gtttctgtgg ggtatgatta tcaccaagcc aaacatcatc atggagtga ggtgaaggaa
 481 gtggagcagg gagaggagcc gtggataatg gaagggtgaat ttccatgtca acatagtcca
 55 541 gaacctgcta aggccatcaa acctattgat cggaagtcag tccatcagat ttgctctggg
 601 ccagtggtag tgagtctaag cactgcagtg aaggagttag tagaaaacag tctggatgct

661 ggtgccacta atattgatct aaagcttaag gactatggag tggatctcat tgaagtttca
721 gacaatggat gtggggtaga agaagaaaac tttgaaggct taatctcttt cagctctgaa
781 acatcacaca tgtaa

5 hPMSL9 (human protein) ACCESSION NM_005395
MCPWRPRLGRRCMVSPREADLGPKDTRLDLPRSPARAPREQNS
10 LGEVDRRGPREQTRAPATAAPPRPLGSRGAEEAEQEGLSATVSACFQEQQEMNTLQG
PVSFKDVAVDFTQEEWRQLDPDEKIAYGDVMLENYSHLVSVGYDYHQAKHHHGVEVKE
15 VEQGEEPWIMEGEFPCQHSPEPAKAIKPIDRKSVHQICSGPVVLSLSTAVKELVENSL
DAGATNIDLKLDYGVDLIEVSDNGCGVEEENFEGLISFSSETSHM"

WE CLAIM:

- 5 1. A method for making a hypermutable yeast, comprising the step of:
introducing into a yeast a polynucleotide comprising a dominant
negative allele of a mismatch repair gene, whereby the cell becomes
hypermutable.
2. The method of claim 1 wherein the mismatch repair gene is a MutH
10 homolog.
3. The method of claim 1 wherein the mismatch repair gene is a MutS
homolog.
4. The method of claim 1 wherein the mismatch repair gene is a MutL
homolog.
- 15 5. The method of claim 1 wherein the mismatch repair gene is a MutY
homolog.
6. The method of claim 1 wherein the mismatch repair gene is *PMS2*.
7. The method of claim 1 wherein the mismatch repair gene is plant *PMS2*.
8. The method of claim 1 wherein the mismatch repair gene is *MLH1*.
- 20 9. The method of claim 1 wherein the mismatch repair gene is *MLH3*.
10. The method of claim 1 wherein the mismatch repair gene is *MSH2*.
11. The method of claim 1 wherein the mismatch repair gene is a *PMSR* or
PMSL homolog.
12. The method of claim 4 wherein the allele comprises a truncation
25 mutation.
13. The method of claim 6 where the allele comprises a truncation
mutation.
14. The method of claim 7 where the allele comprises a truncation mutation.
15. The method of claim 3 where the allele comprises a truncation mutation.

16. The method of claim 3 wherein the allele comprises a truncation mutation at codon 134.
17. The method of claim 4 wherein the allele comprises a truncation mutation at codon 134.
- 5 18. The method of claim 6 wherein the allele comprises a truncation mutation at codon 134.
19. The method of claim 1 wherein the polynucleotide is introduced into a yeast by mating.
20. The method of claim 6 wherein the mismatch repair gene is mammalian
- 10 *PMS2*.
21. The method of claim 14 wherein the mismatch repair gene is plant *PMS2*.
22. The method of claim 12 wherein the mismatch repair gene is *MLH1*.
23. The method of claim 12 wherein the mismatch repair gene is *MLH3*.
- 15 24. The method of claim 15 wherein the mismatch repair gene is *MSH2*.
25. The method of claim 15 wherein the mismatch repair gene is *MSH3*.
26. The method of claim 15 wherein the mismatch repair gene is *MSH6*.
27. The method of claim 12 wherein the mismatch repair gene is a plant MutL homolog.
- 20 28. A homogeneous composition of cultured, hypermutable, yeast which comprise a dominant negative allele of a mismatch repair gene.
29. The isolated hypermutable yeast of claim 28 wherein the mismatch repair gene is a mutL gene or homolog.
30. The isolated hypermutable yeast of claim 28 wherein the mismatch
- 25 repair gene is a *PMS2* gene or homolog.
31. The isolated hypermutable yeast of claim 28 wherein the mismatch repair gene is a *MLH1* or homolog.
32. The isolated hypermutable yeast of claim 28 wherein the mismatch repair gene is a *PMSR* homolog.
- 30 33. The isolated hypermutable yeast of claim 28 wherein the mismatch repair gene is *mutS* or a homolog.

34. The isolated hypermutable yeast of claim 28 wherein the mismatch repair gene is eukaryotic.
35. The isolated hypermutable yeast of claim 28 wherein the mismatch repair gene is procaryotic.
- 5 36. The isolated hypermutable yeast of claim 30 wherein the cells express a protein consisting of the first 133 amino acids of PMS2.
37. The isolated hypermutable yeast of claim 30 comprising a protein which consists of the first 133 amino acids of PMS2.
38. The isolated hypermutable yeast of claim 33 comprising a mammalian
- 10 MutS protein.
39. The isolated hypermutable yeast of claim 31 comprising a protein which consists of a mammalian MutL protein.
40. The isolated hypermutable yeast of claim 28 comprising a eukaryotic MutL protein.
- 15 41. The isolated hypermutable yeast of claim 28 comprising a eukaryotic MutS protein.
42. A method for generating a mutation in a gene of interest comprising the steps of:
- growing a yeast culture comprising the gene of interest and a
- 20 dominant negative allele of a mismatch repair gene, wherein the cell is hypermutable; and
- testing the cell to determine whether the gene of interest harbors a mutation.
43. The method of claim 42 wherein the step of testing comprises
- 25 analyzing a nucleotide sequence of the gene of interest.
44. The method of claim 42 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.
45. The method of claim 42 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.

46. The method of claim 42 wherein the step of testing comprises analyzing a phenotype associated with the gene of interest.
47. The method of claim 42 wherein the yeast is made by the process of introducing a polynucleotide comprising a dominant negative allele of a mismatch repair gene into a yeast cell, whereby the yeast cell becomes hypermutable.
48. The method of claim 47 wherein the step of testing comprises analyzing the nucleotide sequence from the gene of interest.
49. The method of claim 47 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.
50. The method of claim 47 wherein the step of testing comprises analyzing the phenotype of the gene of interest.
51. A method for generating a mutation in a gene of interest comprising the steps of:
- growing a yeast cell comprising the gene of interest and a polynucleotide encoding a dominant negative allele of a mismatch repair gene to create a population of mutated, hypermutable yeast cells;
- cultivating the population of mutated, hypermutable yeast cells under trait selection conditions;
- testing the yeast cells which grow under trait selection conditions to determine whether the gene of interest harbors a mutation.
52. The method of claim 51 wherein the step of testing comprises analyzing a nucleotide sequence of the gene of interest.
53. The method of claim 51 wherein the step of testing comprises analyzing mRNA transcribed from the gene of interest.
54. The method of claim 51 wherein the step of testing comprises analyzing a protein encoded by the gene of interest.
55. The method of claim 51 wherein the step of testing comprises analyzing a phenotype associated with the gene of interest.

56. The method of claim 51 further comprising the step of using the yeast cells which harbor a mutation in the gene of interest to produce a recombinant product.
57. The method of claim 51 further comprising the step of using the
5 yeast cells which harbor a mutation in the gene of interest to perform a biotransformation.
58. The method of claim 51 further comprising the step of using the yeast cells which harbor a mutation in the gene of interest to perform bioremediation.
- 10 59. The method of claim 51 further comprising the step of using the yeast cells which harbor a mutation in the gene of interest to identify genes encoding viral antigens.
60. The method of claim 51 further comprising the step of using the yeast cells which harbor a mutation in the gene of interest to identify yeast
15 antigens.
61. The method of claim 51 further comprising the step of using the yeast cells which harbor a mutation in the gene of interest to identify pharmaceutical targets.
62. The method of claim 51 wherein the mutation in the gene of interest
20 causes antibiotic resistance, and the gene is cloned.
63. The method of claim 51 further comprising the step of using the yeast cells which harbor a mutation in the gene of interest to screen compound libraries.
64. A method for generating enhanced hypermutable yeast comprising the
25 steps of:
exposing a yeast cell to a mutagen, wherein the yeast cell is defective in mismatch repair (MMR) due to the presence of a dominant negative allele of at least one MMR gene, whereby an enhanced rate of mutation of the yeast cell is achieved.

65. The method of claim 64 wherein the mutagen is a DNA alkylating agent.
66. The method of claim 64 wherein the mutagen is a DNA intercalating agent.
- 5 67. The method of claim 64 wherein the mutagen is a DNA oxidizing agent.
68. The method of claim 64 wherein the mutagen is ionizing radiation.
69. The method of claim 64 wherein the mutagen is ultraviolet irradiation.
70. The method of claim 64 wherein the dominant negative allele is inducibly regulated.
- 10 71. A method for generating mismatch repair (MMR)-proficient yeast with new output traits, comprising the steps of:
- growing a yeast cell comprising a gene of interest and a polynucleotide encoding a dominant negative allele of a mismatch repair gene to create a population of mutated, hypermutable yeast cells;
- 15 cultivating the population of mutated, hypermutable yeast cells under trait selection conditions;
- testing the yeast cells which grow under trait selection conditions to determine whether the gene of interest harbors a mutation;
- restoring normal mismatch repair activity to the yeast cells..
- 20 72. The method of claim 71 wherein the yeast cell is exposed to a mutagen to increase the rate of mutation prior to the step of cultivating.
73. The method of claim 71 wherein the step of restoring normal mismatch repair activity comprises removing an inducer which regulates transcription of the dominant negative allele from the yeast cells.
- 25 74. The method of claim 72 wherein the step of restoring normal mismatch repair activity comprises removing an inducer which regulates transcription of the dominant negative allele from the yeast cells.
75. The method of claim 73 wherein the inducer is methanol.
76. The method of claim 73 wherein the inducer is galactose.

77. The method of claim 71 wherein the step of restoring normal mismatch repair activity comprises excising the dominant negative allele by homologous recombination.
78. The method of claim 71 wherein the step of restoring normal mismatch repair activity involves inactivating the dominant negative allele.
79. The method of claim 71 wherein the step of restoring normal mismatch repair activity comprises applying selection conditions to the yeast cells under which cells which have lost the dominant negative allele can grow but cells which harbor the dominant negative allele cannot grow.
80. The method of claim 71 wherein the step of restoring normal mismatch repair activity is performed subsequent to the step of cultivating under trait selection conditions.
81. The method of claim 72 wherein the step of restoring normal mismatch repair activity is performed subsequent to the step of exposing to a mutagen and subsequent to the step of cultivating under trait selection conditions.
82. The method of claim 72 wherein mutagen is ionizing radiation.
83. The method of claim 72 wherein the mutagen is ultraviolet (UV) irradiation.
84. The method of claim 71 wherein normal mismatch repair activity is restored by complementing with a wild-type mismatch repair allele.

INTERNATIONAL SEARCH REPORT

International Application No.

PC1/US 01/05447

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/81 C12N5/10 C12N15/01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, MEDLINE, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2 240 609 A (THE JOHNS HOPKINS UNIVERSITY, US) 14 October 1999 (1999-10-14) the whole document	1-84
P, X	& US 6 146 894 A (NICHOLAS NICOLAIDES ET AL.) 14 November 2000 (2000-11-14) the whole document --- -/--	1,6,8, 10,11, 13,18, 20,28, 30-32, 34,36, 37,42-71

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

26 June 2001

Date of mailing of the international search report

10/07/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040. Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Montero Lopez, B

INTERNATIONAL SEARCH REPORT

International Application No

PCI/US 01/05447

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NICOLAIDES NICHOLAS C ET AL: "A naturally occurring hPMS2 mutation can confer a dominant negative mutator phenotype" MOLECULAR AND CELLULAR BIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, US, vol. 18, no. 3, March 1998 (1998-03), pages 1635-1641, XP002165242 ISSN: 0270-7306 cited in the application abstract page 1636, right-hand column, paragraph 4 -page 1640, right-hand column, paragraph 1 ----	1-84
Y	POLACZEK P ET AL: "Functional genetic tests of DNA mismatch repair protein activity in <i>Saccharomyces cerevisiae</i> " GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, GB, ELSEVIER SCIENCE PUBLISHERS, BARKING, vol. 213, no. 1-2, 15 June 1998 (1998-06-15), pages 159-167, XP004125015 ISSN: 0378-1119 the whole document ----	1-84
A	ALEXANDER ARONSHTAM ET AL.: "Dominant negative mutator mutations in the mutL gene of <i>Escherichia coli</i> " NUCLEIC ACIDS RESEARCH, vol. 24, no. 13, 1996, pages 2498-2504, XP002168605 cited in the application the whole document ----	2-5
A	MARILIA CASCALHO ET AL.: "Mismatch repair co-opted by hypermutation" SCIENCE, vol. 279, 20 February 1998 (1998-02-20), pages 1207-1210, XP002168607 DC the whole document -----	1-84

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/05447

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CA 2240609 A		NONE	

Form PCT/ISA/210 (patent family annex) (July 1992)

